CLINICAL STUDY ON SIDDHA MEDICINE IN THE MANAGEMENT OF "MADHUMEGAM" (TYPE II DIABETES MELLITUS)

THESIS

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DECLARATION BY THE CANDIDATE

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This is to certify that the thesis entitled "**CLINICAL STUDY OF SIDDHA MEDICINE IN THE MANAGEMENT OF MADHUMEGAM-TYPE II DIABETES MELLITUS**" submitted for the degree of Doctor of Philosophy in Siddha by Dr.K.Ravi is the record of research work carried out by him during period of 2011-2015 at The Tamil Nadu Dr.M.G.R Medical University Chennai and National Institute of Siddha , Chennai under my guidance and supervision and this work has not formed the basis for the award to the candidate of any degree, diploma, associate-ship, fellowship or other similar titles represents the independent work done by his under my supervision and guidance.

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List of Abbreviations

WHO	- World health organization
ISO	- International Organization for standardization
OECD	- Organization for Economic Co-operation and Development
AYUSH	- Ayurveda Yoga Unani Siddha Homeopathy
GMP	- Good Manufacturing Practice
SGPT	-Serum Glutamate Pyruvate Transaminase
SGOT	- Serum Glutamate Oxalo acetic Transaminase
CI	-Confidence Interval
SD	- Standard Deviation
SEM	-Standard Error Mean
STZ	- Streptozotocin
TLC	- Thin layer chromatography
HPTLC	- High performance thin layer chromatography
GC-MS	- Gas chromatography – Mass Spectrometry
FTIR	- Fourier Transform infrared Spectrometry
XRF	-X-ray Fluorescence Spectrometer
ANOVA	- Analysis of Variance
µm	-micrometer
nm	-nanometer
v/v	- Volume by volume
m.v	-Mass by volume
hr	-hours
ppm	-parts per million
LFT	- Liver Function Test
RFT	- Renal Function Test
HbA_1C	-Glycated Haemoglobuln

b.w	-Body Weight
g	- Gram
l	- Liter
ml	- milliliter
µl	- microliter
dl	- deci liter
ng	- nanogram
mg	-milligram
µg	-microgram
kg	- kilogram
Cumm	- cubic millimeter
Cm	- centimeter
FBS	- Fasting Blood Sugar
PPBS	- Post Prandial Blood Sugar
FUS	- Fasting Urine Sugar
PPUS	- Post Prandial Urine Sugar
RBS	- Random Blood Sugar
ICP-OES	- Inductive coupled Plasma Optical Emission Sprectroscopy
CPSCEA	- Committee for the Purpose of Control and Supervision of Experiments on Animals
RI	- Retention Time
NIST	- National Institute of Standard and Technology
RI	- Retention Indices
IR	- Infrared
HV	- High Vacuum

LV	- Low Vacuum
ESEN	- Environment Staining Electron Microscope
MTD	- Maximum Tolerated Dose
NVK	- Nila Vembu Kudineer
NOEL	- No Observed Effect Level
NA	-Not Applicable

CHAPTER I

Introduction

1.1 Introduction

In the past decades, diabetes mellitus has become a major health problem world wide, reaching epidemic proportions in many developing countries including India. **World wide projections** suggest that >220 million people will have diabetes by the year 2020 and majority of these (approximately 213 million) will have type-II diabetes. Recently Diabetes mellitus has emerged as a leading metabolic disorder. India is the diabetic capital of the world, with 41 million people affected with the disease.(Ranjith Unnikrishnan., et al 2007)

It is the most prevalent disease in the world affecting 25% of population and afflicts 150 million people and may raise to 300 million by 2025. Diabetes mellitus is considered as one of the five leading cause of death in the world. **India with largest** diabetic population is expected to have 57.2 million diabetic patients by the year 2025. The National Urban Diabetes study (NUDS) was recently conducted in six major cities covering all regions of the country. In each of the cities studied, prevalence of diabetes is higher than 9% ranging from 9.3%-16.6%. The average diabetic prevalence in urban Indian adults is 12.1%. prevalence of diabetes in rural area is 4 to 6 times lower than in the cities.(Azad Khan et al 1995)

Recently WHO and International Diabetic Federation (IDF) reports show that the incidence of diabetes is increasing at an epidemic rate, especially in developing countries, probably due to rapidly changing life style, eating habits and environmental conditions. India has been projected by WHO as the country with fastest growing population of diabetic patients. It is estimated that between 1995-2025 diabetic patients in India will increase by 195%.(Chatterje, M.N. and Rana Sinde 1995)

1.2Global Scenario

The overwhelming burden of the disease continues to be shouldered by low- and middle- income countries where four out of five people are living with diabetes. The burden of diabetes is reflected not only in the increasing numbers of people with diabetes, but also in the growing number of premature deaths due to diabetes. In 2013, approximately half of deaths are due to diabetes in adults under the age of 60 and in less-developed regions like

sub-Saharan Africa, that proportion climbed to 75%. Globally diabetic population is increasing at an alarming rate **(Cho *et al*., 2013)**.

1.3 Indian Scenario

India with its dubious distinction of being called, "the diabetic capital of the world" is presently estimated to have over 30 million individuals suffering with this deadly disease. The prevalence of diabetes in India is over 12 %. It is estimated that there are 30 million diabetics in India today. India is ahead of China and USA, which are in second and third place, respectively.

To certain extent geographical distribution influences the pattern of diabetes incidences across India **(Kaveeshwar and Cornwall, 2014).** The results of a study conducted by the Indian Council of Medical Research (ICMR) revealed that the states of Northern India ((Chandigarh 0.12 million, Jharkhand 0.96 million) had a lower incidence of diabetes unlike Maharashtra (9.2 million) and Tamil Nadu (4.8 million) **(Anjana *et al*., 2011)**. These results corroborated with the findings of The National Urban Survey conducted across the metropolitan cities of India whose results showed a statistical report of 11.7% diabetes incidence in Kolkata (Eastern India), 6.1% in Kashmir Valley (Northern India) **(Zargar *et al*., 2000),** 11.6% in New Delhi (Northern India), and 9.3% in West India (Mumbai) as compared to 13.5% in Chennai South India, 16.6% in Hyderabad (south India), and 12.4% in Bangalore (South India) (**Ramachandran *et al*., 2001**).

The overburdened healthcare system has difficulty in providing means of glycemic control to the millions of patients. This failure today may lead to a future onslaught of complications, particularly cardiovascular complications, which further challenge the resources of the Indian health-care system

1.4TYPES OF DIABETES MELLITUS

Type 1 Diabetes mellitus (DM) results from the pancreatic failure to produce enough insulin. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". The cause is unknown.

Type 2 Diabetes mellitus begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the disease progresses lack of insulin also develops. This form was previously referred as "non-insulin-dependent diabetes mellitus" or "adult-onset diabetes". The primary cause is excessive body weight and lack of exercise.

Gestational diabetes, is the third main form and occurs when pregnant women without a previous history of diabetes develop a high blood sugar level.

1.5 Signs and Symptoms

The classic symptoms of untreated diabetes are weight loss, polyuria polydipsia and polyphagia that may develop rapidly in type 1 diabetes, while they usually develop much more slowly and may be subtle or absent in type 2 diabetes. Several other signs and symptoms can mark the onset of diabetes, although they are not specific to the disease. In addition to the known ones as described above, blurry vision, headache, fatigue, slow healing of cuts, and itchy skin, are the other symptoms often seen in diabetic patients. Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to changes in its shape, resulting in vision changes. A number of skin rashes that can occur in diabetes are collectively known as diabetic dermatomes.

Prevention and treatment involve a healthy diet, physical exercise and avoiding tobacco. Blood control and proper foot care are also important for people with the disease. Type 1 diabetes is managed with insulin injections. Type 2 diabetes is often treated with medications with or without insulin. Insulin and some oral medications can cause low blood sugar. Weight loss surgery in those with obesity is sometimes an effective measure in people with type 2 DM. Gestational diabetes usually resolves after the birth of the baby. As of 2014, an estimated 387 million people have diabetes worldwide, with type 2 diabetes making up about 90% of the cases. This represents 8.3% of the adult population, with equal rates in both women and men. From 2012 to 2014, diabetes is estimated to have resulted in 1.5 to 4.9

million deaths each year. Diabetes at least doubles a person's risk of death. The number of people with diabetes is expected to rise to 592 million by 2035. The global economic cost of diabetes in 2014 was estimated to be $612 billion USD. In the United States, diabetes cost $245 billion in 2012.(Amy Aronouitz et al.,2006)

1.6 Type 2 Diabetes Mellitus

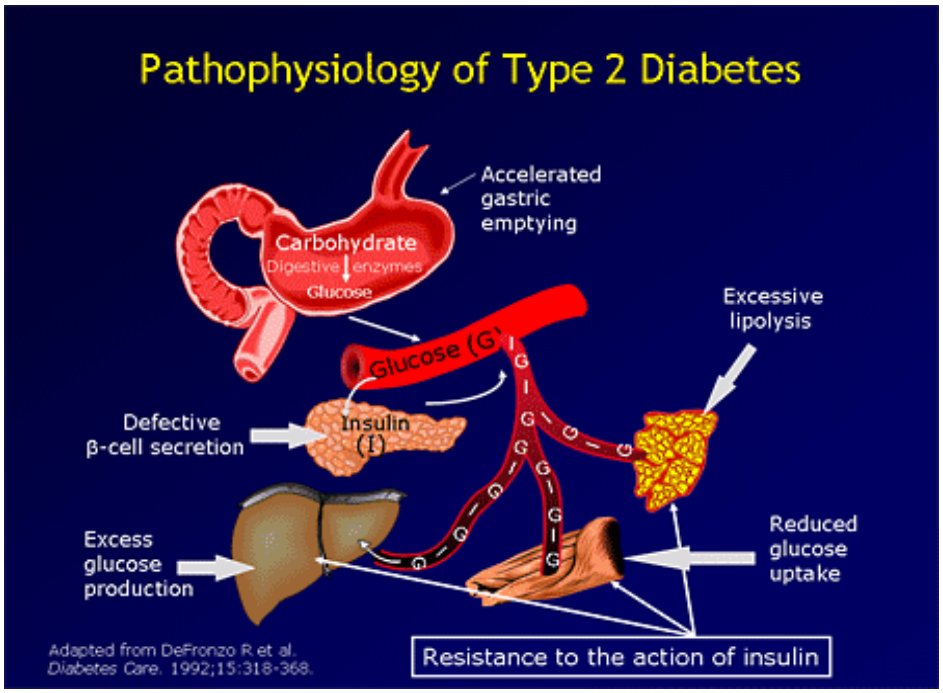
Type 2 diabetes mellitus is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus cases due to a known defect are classified separately. Type 2 diabetes is the most common type.

In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity. At this stage, hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver. Type 2 diabetes is due primarily to lifestyle factors and genetics. A number of lifestyle factors are known to be important for the development of type 2 diabetes, including obesity (defined by a body mass index of greater than thirty), lack of physical activity, poor diet, stress, and urbanization. Excess body fat is associated with 30% of cases in those of Chinese and Japanese descent, 60–80% of cases in those of European and African descent, and 100% of Pima Indians and Pacific Islanders. Even those who are not obese often have a high waist–hip ratio. Dietary factors also influence the risk of developing type 2 diabetes. Consumption of sugar-sweetened drinks in excess is associated with an increased risk. The type of fats in the diet is also important, saturated fats and trans fatty acids increase the risk of diabetes and polyunsaturated and monounsaturated fat decrease the risk. Eating lots of white rice also appears to play a role in increasing the risk. Lack of exercise is believed to cause 7% of cases.(Agarwal,2003)

1.7 Gestational Diabetes

Gestational Diabetes Mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2–10% of all pregnancies and may improve or disappear after delivery. However, after pregnancy approximately 5–10% of women with gestational diabetes are found to have diabetes mellitus, most commonly type 2.Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. Management may include dietary changes, blood glucose monitoring, and in some cases insulin may be required. Though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital cardiac and central nervous system anomalies, and skeletal muscle malformations. Increased fetal insulin may inhibit fetal surfactant production and cause respiratory distress syndrome. A high blood bilirubin level may result from red blood cell destruction. In severe cases, perinatal death may occur, most commonly as a result of poor placental perfusion due to vascular impairment. Labor induction may be indicated with decreased placental function. A Caesarean section may be performed if there is marked fetal distress or an increased risk of injury associated with macrosomia, such as shoulder dystocia.(Robbins 1994)

Mechanism of insulin release in normal pancreatic beta cells — insulin production is more or less constant within the beta cells. Its release is triggered by food, chiefly food containing absorbable glucose.



Insulin is the principal hormone that regulates the uptake of glucose from the blood into most cells of the body, especially liver, muscle, and adipose tissue. Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus. The body obtains glucose from three main places: the intestinal absorption of food, the breakdown of glycogen - the storage form of glucose found in the liver, and gluconeogenesis - the generation of glucose from non-carbohydrate substrates in the body. Insulin plays a critical role in balancing glucose levels in the body. Insulin can inhibit the breakdown of glycogen or the process of gluconeogenesis, it can stimulate the transport of glucose into fat and muscle cells, and it can stimulate the storage of glucose in the form of glycogen. Insulin is released into the blood by beta cells (β-cells), found in the islets of Langerhans in the pancreas, in response to rising levels of blood glucose, typically after eating. Insulin is used by about two-thirds of the body's cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage. Lower glucose levels result in decreased insulin release from the beta cells and in the breakdown of glycogen to glucose. This process is mainly controlled by the hormone glucagon, which acts in the opposite manner to insulin.

If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or insulin resistance), or if the insulin itself is defective, then glucose will not be absorbed properly by the body cells that require it, and it will not be stored appropriately in the liver and muscles. The net effect is persistently high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis. When the glucose concentration in the blood remains high over time, the kidneys will reach a threshold of reabsorption, and glucose will be excreted in the urine (glycosuria). This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells and other body compartments, causing dehydration and increased thirst (polydipsia).

1.8Diabetic Complications

The long-standing medical complications of DM include retinopathy, nephropathy, peripheral neuropathy (nerve disease), and heart disease/stroke (**Leese *et al*., 1992).**

1.9 Diabetic Retinopathy

Diabetic retinopathy is characterized by abnormalities of small blood vessels in the retina caused by DM. The end result is the weakening or leakage of these blood vessels, and subsequent bleeding into the fluid-filled center of the eye (ADA, 2001; **Clayman, 1994**). Diabetic retinopathy is the leading cause of blindness in individuals between 20-74 years of age, with approximately 12,000 to 24,000 individuals becoming blind each year. DM is responsible for 8% of legal blindness in the United States (ADA, 2001).

1.10 Diabetic Microangiopathy

One of the most consistent morphologic features of diabetes is diffused thickening of basement membranes **(Tisher *et al.*, 1994)**. The thickening is most evident in the capillaries of the skin, skeletal muscles, retina, renal glomeruli and renal medulla, giving rise to the characteristic diabetic microangiopathy of these organs. However, it may also be seen in such nonvascular structures as renal tubules, Bowman's capsule, peripheral nerves and placenta. The microangiopathy is clearly related to hyperglycemia. A number of biochemical basement membrane alterations occur, including increased amounts and, synthesis of collagen type IV

and decrease in proteoglycans. The latter can account for the increased glomerular permeability characteristic of diabetic nephropathy.

1.11Diabetic Nephropathy

Nephropathy (kidney disease) is a common complication of DM. Diabetic nephropathy is a progressive disease, and is the leading cause for end-stage renal disease (ESRD) **(Leese, 1992)**. Diabetic nephropathy is characterized by damaged and leaky blood vessels in the kidneys. Eventually, the entire filtration system becomes destroyed, and the kidneys fail to function. This is called end-stage renal disease (ESRD). Approximately 40% of ESRD is due to DM complication **(ADA, 2001)**.The prevalence of micro albuminuria and macro albuminuria was significantly higher in patients whose diabetes had developed before rather than after the age of 20.

1.12Diabetic Neuropathy

Peripheral neuropathy occurs when there is damage to the peripheral nerves that lead from the brain and spinal cord out to the rest of the body. Symptoms include tingling and numbness of the extremities. Unawareness of injury or infection to the extremities, as well as muscular atrophy, can result in amputation. Approximately 60-70 % of individuals with DM have some form of nerve damage. DM is the most frequent cause of non-traumatic lower limb amputations **(Leese, 1992)**, with individuals with DM being 15-40 times at greater risk for leg amputation **(ADA, 2001)**. People with DM run a higher risk of developing atherosclerosis, along with risks of high blood pressure, heart attack, and stroke **(ADA, 2001; Leese, 1992)**. Heart disease is present in 75% of diabetes-related deaths, and individuals with DM are 2 to 4 times more likely to suffer a stroke **(ADA, 2001)**.

Table:1 Oral Anti-Diabetic Drugs and their Side Effects

Drugs	Side Effects
Sulfonylureas	Hypoglycemia and usually minor skin allergy.
Meglitinides	Hypoglycemia and minor symptoms are skin rash or itching; gastro-intestinal upset.
Biguanides	The medication may cause swelling and increased weight
Thiazolidinediones	Fluid accumulation becomes a serious problem
Alpha-glucosidase-	Impairment of vital organs like liver results in less annoying side effects flatulence or diarrhea.
Dipeptidyl Peptidase-IV Inhibitors	The headache, inflammation of nose and throat, joint problem and painful extremities
D-Phenylalanine Derivatives	Has a risk of lowering the blood glucose levels
Short Acting Insulin- GLP-1 Analogs	mild & mainly GI tune time (nausea in 44%; vomiting &diarrohea)
Amylin Analogs	Produces side effects leading to impairment in vision speech besides being producing nervous weakness and palpitations

1.13 Need For Alternative Therapy

It is often observed that longer administration of existing synthetic oral hypoglycemic agents and insulin are prone to influence blood parameters, gastrointestinal conditions leading to hypoglycemic change resulting in losing conscience and disturbing the functions of vital organs. Besides the agents cannot be used during pregnancy.

Even though numerous drugs targeted for carbohydrate hydrolysing enzymes (pseudosaccharides), release insulin from pancreatic b-cells (sulphonyl urea), glucose utilization (biguanides), insulin sensitizers, PPAR gamma agonists (glitazones) are also in clinical practice and the rising diabetes market observes a number of changes (**Ashok and Tiwari, 2002**). The glitazones are intended to address the problem of insulin resistance and increase insulin action at the cellular level; nevertheless, various drugs are associated with liver toxicity (troglitazone), plus mortality due to hepatic failure **(Krische *et al*., 2000; Stern *et al*., 1999**) and raising the symptoms and risk factors for heart disease and heart failure (rosiglitazone) (**Gale *et al*., 2001**). As the long-term effects on the complications of diabetes related to drugs are not yet clear, UK Drug and Therapeutic Bulletin warrants that patients taking glitazones be monitored for signs of heart failure (**Ashok and Tiwari, 2002**).

In spite of greater advancements made in understanding and managing this disease, the graph of diabetes-related complications and mortality are rising unabatedly. Traditional medicine - basically plants with various active principles; and therapeutic properties have been used since time immemorial by physicians and laymen to treat a great variety of human diseases including diabetes.

1.14 Indian System of Medicine

Every country in this world is having a system of its own traditional medicine. The strong tradition of health care practiced and documented for more than 3000 years in India is in the form of Siddha, Ayurveda, Unani, and Yoga & Naturopathy systems of medicine. These systems have evolved through centuries of usage and have stood the test of time in providing holistic health care to the people and are based on a very intimate and well researched understanding that biotic and non-biotic components of the environment play a role in promoting holistic health care.

Among the traditional systems of medicine existing in India Ayurveda and Siddha are most popular. The Siddha system of medicine is the oldest and is derived from the vegetable mineral and animal kingdom. Siddha system which has flourished in the South and Ayurveda, is prevalent in the North. These practices deal, not merely with the body of man but also with the inner soul. The art of medicine is based on truth and as such it is a divine art not to be adulterated for the purpose of money.

1.15Ayurvedic perspectives of Diabetes Mellitus

Ayurveda mentions there are 20 types of Diabetes Mellitus 10 are born from slesma (*Kapha*) 6 from *pitta* and 4 from anila (*Vata*)

Table: 2 Classification of Prameha according to different ayurvedic Acharyas.

Charaka	Sushruta	Vagbhata
Kaphajameha		
Udakameha	Udakameha	Udakameha
Ikshuvalikameha	Ikshuvalikameha	Ikshumeha
Sandrameha	Sandrameha	Sandrameha
Sandraprasadmeha	Pishtameha	Pishtameha
Siktameha	Siktameha	Siktameha
Shitameha	Lavanmeha	Shitameha
Shuklameha	Surameha	Surameha
Shukrameha	Shukrameha	Shukrameha
Alalmeha	Phenameha	Lalameha
Shanaimeha	Shanaimeha	Shanaimeha
Pittajameha		
Ksharameha	Ksharameha	Ksharameha
Kalameha	Amlameha	Kalameha
Nilameha	Nilameha	Nilameha
Lohitameha	Shonitameha	Raktameha
Manjishthameha	Manjishthameha	Manjishthameha

Haridrameha	Haridrameha	Haridrameha
Vatajameha		
Vasameha	Vasameha	Vasameha
Majjameha	Sarpimeha	Majjameha
Hastimeha	Hastimeha	Hastimeha
Hastimeha	Kshaudrameha	Madhumeha

Ayurveda states 10 kinds of diabetes produced by *kapha* are curable, 6 kinds produced by *pitta* are controllable but the 4 kinds produced by *vatta* are incurable. Many herbs such as turmeric, ghooseberry are mentioned in the literature to be useful in the management of diabetes.

In the present dissertation **Siddha system and Siddha formulations** used in the management of Diabetes will be focused.

1.16 Siddha System of Medicine

Siddha system is one of the oldest systems of medicine in India. The Siddha system of medicine has developed on the basis of Saiva siddhanda philosophy and Dravidian culture. The word 'Siddha' comes from the root word 'Siddhi' which means 'an object to be attained' or 'perfection' or 'heavenly bliss'. Siddhi generally refers to the eight supernatural powers enumerated as 'anima' and those who have attained them are called 'Siddhars'.

Medicine means the prevention of physical illness;
medicine means the prevention of mental illness;
prevention means to avert illness;
medicine therefore is the prevention of death.

This quotation is an interesting definition of medicine by Tirumoolar -one of the greatest and earliest Tamil Siddhar. Lot of authors assume that “The Tamil Siddhars” invented or developed the Tamil medical system named Siddha medicine. These worshipful Tamil siddhars using their divine powers had classified 4,448 diseases and prescribed medicines with real therapeutic value in the form of herbs, roots, salts and minerals.

The Siddha system basically cures the soul and then the body, the same idea is referred by Thirumoolar in his writings Thirumandram,

Udamparr aziyin uyrirr azhivar
Thidambada Meignanam seravum matter
Udambaivalarkumubayamarinthe
Udambaivalarthanuyirvalarthana

In siddha disease is classified based on the Panchabootha theory and Tridosha theory. *Vadham, Pitham* and *Kapham* are the three humours which are the life constituents of the human body. Still, there is predominant *Vadham*, below the umbilicus, predominant *Pitham* in the abdomen and thorax region and predominant *Kapham* in the head and neck region. *Vadham* plays a vital role to assist the body functions. *Pitham* is responsible for digestion, vision, maintenance of body temperature, hunger, thirst taste etc. Pallor, indigestion, deep sleep, sweet taste in the tongue are functions of *Kapham*.

In siddha system of medicine, great emphasis is given to herbs by ancient Siddhars; that is stressed in the Tamil poem as follows:

"*Veru paru thazhai paru minginikal*

"*Mella mella parpam chenduram paru*

The treatment plan is to regularize the deviated *dosha*, to strengthen the *thathus* and to balance the distribution of *Prana*. *Siddha* aims at bringing the three *doshas* to equilibrium. In the treatment of a disease first suggestion is to treat with herbs, followed by mineral preparations if necessary.

The, administration of *parpam* and *chenduram*, were identified by five properties. One is the *suvai* or the taste, the *guna* or the character, the *veeriya* or the potency, the *pirivu* or class and *mahimai* or action. All these five properties are based on the five elements theory that is present in a drug.

1.17 Diabetes in Siddha System of Medicine

Modern terminology Diabetes mellitus is known as *Madumegam* in Siddha system of medicine. *Madumegam* is popularly known as *Neerizhivu* as per Siddha system of medicine. The term D*iabetes Mellitus* is recognized as *'Madhumeha'* in primeval times. Our ancient *Siddha* Physicians have mastered the science of managing this disorder with effective balance of *'dosha'* using some herbs or plant food sources as medicine and following strict dictary regimen (Pathyam). Those indigenous foods alone may not be as effective as insulin in lowering the blood sugar but the combination therapy seems to equate with the modern methods of drug, diet and exercise. The disease according to the *Siddha* literature is due to the vitiation of *kapha* humour which results in detoriation of seven *udalkattukal* (*saram, sennir, oon, enbu, koluppu, moolai, sukkilam*) and derangement of 14 *vegangal*, ten *vayus* and three *doshas*.

Siddha has been the first to give an elaborate description of this disease, its clinical features, patterns and its management by diet, exercise and herbal or herbo mineral drug. More emphasis is laid on the prophylactic and curative measures and not merely on symptomatic treatment. The Siddha concept of management of *Madhumeham* (diabetes) is still recognized specifically due to its potential, ready availability and lack of toxicity and side-effects.

Siddha medicine advocates the removal of the root cause of the disease which causes the onset of diabetes. It is of primary importance. Secondly it is bringing up of three *doshas* and seven *thathus* in order. Thus, to set right the seven *thathus* which are thrown out of order due to the vitiation, treatment of diabetes is based on maintaining a normal equilibrium of *doshas* and the body with enough energy to function properly.

1.18 Aetiology (NOI VARUM VAZHI)

Superficially this disease is the result of mal functioning of pancreas. The ancient works of Siddha, attribute this disease to excessive intake of food rich in carbohydrates, fat and non-vegetarian food, excessive indulgence of sex, worry, tension, laziness and sedentary jobs.

1.19 Symptoms (KURIGUNANGAL)

The principle symptoms of *neerizhivu* are poly urea, intense thirst (polydypsia) and increased appetite (poly phagia), passing urine slightly yellow in color, pain in the external genital organs, loss of weight and pale complexion. If the urine is heated, the sweet odour will be percepted, white sediments are also observed. Other symptoms are pain all over the body, dysponea and excessive sweating with bad odour, giddiness, vomiting, tastelessness and sleeplessness.

The urine may or may not contain sugar. In the former case, it is called *Madhumegam* (Diabetes Mellitus) and in the latter it is known as *Athimoothram* (Diabetes Insipidus). In the case of Diabetes mellitus, the patient's skin becomes dry and bowels irregular. The patient also suffers from acute burning sensation of the body. As the disease becomes chronic, pulse becomes feeble; he or she develops pain in the loins and on the sides. The appetite slowly decreases. The feet begins to swell, women experience intense itching in genitals and men suffer from balanitis. The patients also tend to suffer from cataract, diabetic coma and carbuncle.

1.20 Classification (NOI ENN)

Twenty varieties of *meha* disorders have been discussed by *Yugimuni*, *Agasthiar* and *Theraiyar*. The *Yugi* has classified *Meganoi* on the basis of color, consistency, taste, smell and weight etc., Out of the twenty kinds of *megam*, four are caused by *vali*, six are caused by azhal and the remaining ten are caused by Iyyam. Each author who have dealt mega disorders have differently classified them under three *doshas* and have given names according to his concept. But the number, Signs and Symptoms of the classified disorders are almost identical in the description of the disease. Classification of *Meganoi* described in various texts in Indian medicine are as follows:

Yugimuni Vaithiyachinthamani 800

Varieties under *Vatha*

1) Nei mana neer

2) Pasumana neer

3) Oon mana neer

4) Seel neer

Varieties under *Pittha*

1) Yani koluppumanna neer
2) Kattralai manna neer
3) Sunna manna neer
4) Enippu megam
5) Palingu neer
6) Myatkuruthi neer

Varieties under *Kabha*

1) Vasa neer
2) Theli neer
3) Moolaiurukku neer
4) Ela neer
5) Kal neer
6) Sukkila neer
7) Then neer
8) Upppu neer
9) Kalu neer
10) Eraichi neer

1.21 Complications (AVATHTHAIKAL)

The onset of the following sufferings will follow gradually if the disease is not controlled or untreated.

1. Obesity and inflammation in the urinary tract.
2. Frequent micturition, diminution of semen's density and loss of complexion.
3. Dryness in tongue and flatulent abdomen.
4. Severe thirst and abnormality, delirium.
5. Increased quantity of urine and loss of semen.
6. Difficulty in breathing and sleeplessness.
7. Vomiting sensation, tastelessness and general weakness.
8. Formation of abscess or carbuncles.
9. Irregularities in daily habit, bed ridden and bed sores and uncontrolled motion.
10. Secondary disease like TB or some other complications develop and finally lead to death.

1.22 Diagnosis (NOI KANIPPU)

According to the Siddha system, diagnosis of any disease is being done through eight fold examination (*Naa, Niram, Mozhi, Vizhi, Sparisam, Malam, Moothram* and *Nadi*) and confirmation through interrogation.

Naa (Tongue): It remains dry and at times black.

Niram (Colour): It is different from the original complexion and dryness on the skin.

Mozhi (Speech): Due to increase of Azhal, the patient is likely to suffer from giddiness and hence, there is bound to be diffculty in speech.

Vizhi (Eye): Dullness in sight and cataract of the eye is observed.

Malam (Motion): When vali is in high proportion there is constipation. With the increase of Azhal there exists diarrohea and increased Iyyam in white, milky motion.

Moothiram (Urine): When Iyyam is in excess, urine is whitish, foamy and appears to have sediments. When Azhal increases, it is yellow and sweet enough to attract flies. With the increased vali, it resemble ghee with sweet taste.

Nadi (Pulse): The most important parameter of diagnosis is Nadi. In the developed stage of this disease, the Vali, azhal, Iyyam nadi will be feeble. Diabetes is diagnosed with the enhanced speed of Azhal nadi.

1.23 Methods of urine examination: Neerkuri and Neikuri

Collection of urine

Prior to the day of urine examination, the patient should take a balanced diet and rest. The first urine is collected in a glass container.

The colour of urine is noted and a drop of gingelly oil is added to the container and the tendency of spread is noted within 1 ½ hour.

Neikuri

A drop of gingelly oil is added in to a wide vessel containing the urine to be tested and kept under the sunlight. The variations of 3 uyir thathu in disease can be diagnosed by the behavior of gingelly oil on the surface of urine.

By the careful examination of the urine with gingelly oil, the physicians can know whether the disease is curable or not. For this purpose, siddhars explained various spreading tendencies of oil on urine surface to define the prognosis of disease.

1.24 Humoural Pathology (MUKKUTRA VERUPADUGAL)

As referred earlier, disease is always due to the imbalance in the ratio of *Vali, Azhal* and *Iyyam*. This imbalance affects the *Keelnokunkal*, seven *Udalkattukal* and slowly affects the appetite. An imbalance in *Iyyam* does imply an imbalance in the other two Kuttrams too,

and also contribute destruction of the system. But all the types of diabetes if left untreated at the initial stages will develop in to Diabetes mellitus which is incurable. As per textual reference, on examination of pulse (nadi) it could be observed that vitiated Kapha humour joined with vatha humour and pittha humour, combined together contribute further destruction of the system.

1.25 Management of Diabetes by Siddha System of Medicine

The need of the hour is to identify and develop innovative plant based therapies to combat diabetes, and provide evidence – based plant medicine, and prove the advantages of these medicines over the existing therapies. At present globally there is resurgence in the usage of plant-based medicines used in traditional systems such as Siddha and WHO also recommends the same because of its holistic approach with maximum therapeutic potency and minimum side effects. Unfortunately, despite the apparent supremacy in terms of multiple therapeutic efficacies of herbal based medicines, well- organized, rigorous clinical trial evidences are not adequately available. Considering these, it is necessary to provide an alternative solution to counter the diabetic plague through indigenous herbal resources used by Siddha practitioners.

Hence, based on the extensive review of Siddha literature attempts have been made in the present work to evaluate scientifically an anti-diabetic Siddha plant drug *Nilavembu Kudineer*. The present study is focused on studying the effect of *Nilavembu Kudineer* in Streptozotocin (STZ) induced diabetic rats and also to assess its clinical efficacy in Type 2 diabetes mellitus patients. Besides the selected Siddha herbal formulation is also studied from chemical and botanical standardization point of view in order to contribute to the suffering diabetic population **a quality standard anti diabetic Siddha herbal drug.**

CHAPTER II

REVIEW OF LITERATURE

2.1 Medicinal Plants and Diabetes

In recent years, evidence of cases of "insulin resistance" and the occurrence of side effects due to prolonged administration of conventional drugs have triggered the search for safe and effective alternatives. Several plant extracts and isolated phytochemicals have been evaluated for anti-diabetic activity with a view to identify alternative treatment strategies for diabetes. It has been observed that certain resistant cases of diabetes that do not respond well to conventional drugs often respond well to supplementation with natural remedies **(Campbell *et al.*,1997).**

India has one of world's richest medicinal plant heritages. The wealth is not only in terms of the number of unique species (6160) documented thus far for their medicinal use but also in terms of the tremendous depth of traditional knowledge about their uses in human & livestock health and also in agriculture. Over 4786 ecosystem specific species of plants are used by ethnic communities for human and veterinary health care, across the various ecosystems from Ladakh in the trans-Himalayas to the southern coastal tip of Kanyakumari and from the deserts of Rajasthan and Kachch to the hills of the Northeast. Codified medical systems of Ayurveda, Siddha and Unani have enumerated around 2400 unique species of plants that are fully documented in terms of their biological properties, actions and drug formulations for a wide range of health conditions (**Dharsansankar, 2006)**

In recent years, the popularity of complementary medicine has increased. It is observed that dietary measures and traditional plant therapies as prescribed in Ayurvedic and other indigenous systems of medicine have been used by the majority of the population. Surveys conducted in Australia and US indicate that almost 48.5 and 34% of respondents had used at least one form of unconventional therapy including herbal medicine **(Eisenberg *et al.*,1993; Maclennan *et al.*,1996).**

2.2 Reported Anti-Diabetic Plants and their mechanism

In the present dissertation with a view to develop a human friendly anti diabetic herbal drug, a survey was conducted on herbs and herbal formulation used in the management of diabetes. From the review it is noted that many herbs are enriched with anti diabetic potentials and their mechanism of anti diabetic action is also evaluated by various researchers. (Table.1)

Table: 3 Reported anti diabetic plants with the mechanism of anti diabetic action

Sl. No	Botanical Name	Mechanism of action	References
1	*Annona squamosa* L.	Hypoglycemic and antioxidant	Kaleem *et al.,* (2006)
2	*Artemisia pallens* Wall. ex. Dc.	Anti-hyperglycemic and hypolipidemic effect	Subramanian *et al.,* (1996)
3	*Azadirachta indica* A. Juss.	Blocks the inhibitory effect of Serotonin and epinephrine on insulin secretion mediated by glucose.	Biswas *et al.,* (2002)
4	*Beta vulgaris* L.	Initiates insulin release from pancreatic beta cells	Yoshikawa *et al.,*(1996)
5	*Boerhavia diffusa* L.	Reduces insulin resistance	Pari and Satheesh (2004)
6	*Bombax ceiba* L.	Hypoglycemic and hypolipidemic effect	Saleem *et al.,*(1999)
7	*Caesalpinia bonducella* (L.) Fleming	Antioxidant and anti-hyperglycemic effect	Chakrabarti *et al.,* (2005)

8	*Camellia sinensis* (L.) O. Kuntze	Antihyperglycemic and antioxidant effect	Gomes *et al.,* (1995)
9	*Capparis decidua* (Forsk.) Edgew.	Hypoglycemic, hypolipidemic, inhibit alpha amylase activity, and antioxidant effect. Altered level of insulin receptor and GLUT-4 mRNA in skeletal muscle	Agarwal and Chauhan (1988)
10	*Catharanthu sroseus*(L.) G. Don.	Stimulates insulin release from islets of Langerhans.	Marles and Farenworth (1995)
11	*Croton klozhians* L.	Concentration depent increase in insulin secretion in MIN6 cells and improve lipid parameters in diabetic rats	Govidarajan *et al.,*(2008)
12	*Cuminum cyminum* L.	Inhibits aldose reductase and alpha glucosidase activity in experimental animals	Lee (2005)
13	*Emblica officinalis* Gaertn.	Acute induction of insulin release from Beta cells of islet of Langerhans, antihyperlipidemic, hypoglycemic and	Jose and Kuttan (1995)

		antioxidant effect	
14	*Eugenia uniflora* L.	Stimulates insulin release from islets of Langerhans	Arai *et al.*,(1999)
15	*Enicostemma littorale* Blume	Hypoglycemic and antihyperglycemic effect	Maroo *et al.*, (2003)
16	*Gymnema sylvestre* R.Br.	Hypoglycemic effect	Preuss *et al.*,(1998)
17	*Hibiscus rosa-sinensis* L.	Anti-hyperglycemic effect	Sachadeva and Khemani (1999)
18	*Ipomoea batatas* (L.) Lam.	Hypoglycemic and hypocholesterolemic effect	Kusano and Abe (2000)
19	*Momordica cymbalaria* Hook. f.	Hypoglycemic and antioxidant effect	Rao *et al.*,(1999)
20	*Musa sapientum* L.	Anti-inflammatory effect	Pari and Umamahaswari (2000)
21	*Phaseolus vulgaris* L.	Initiates insulin release from pancreatic beta cells	Tormo *et al.*,(2004)
22	*Psidium guajava* L.	Inhibits alpha glucosidase activity	Wang *et al.*, (2007)
23	*Pterocarpus marsupium* Roxb.	Stimulates insulin release from isolated beta islets	Ahmed *et al.*,(1991)
24	*Punica granatum* L.	Reduces insulin resistance and Enhancing sensitivity by insulin	Huang *et al.*,(2005)

		receptor.	
25	*Scoparia dulcis* L.	Hypoglycemic, increases glycogenesis and decreases gluconeogenesis and glycogenolysis	Pari and Latha (2005)
26	*Stevia rebaudiana* (Bertoni) Bertoni	Glucose lowering activity by hepatic gluconeogenesis	Ferreria *et al.*,(2006)
27	*Syzygium alternifolium* Walp.	Hypoglycemic, hypolipidemic, inhibit alpha amylase activity, antioxidant. Altered level of insulin receptor and GLUT-4 mRNA in skeletal muscle	Rao and Rao (2001)
28	*Tinospora crispa* (L.) Hook. f. &Thoms	Insulin-secretagogue activity, antihyperlipidemic, hypoglycemic, antioxidant effect	Noor and Ashcroft (1998)
29	*Vaccinium angustifolium* Aiton	Enhances the uptake of glucose in differentiated C2C12 muscle cells and 3T3-l1 adipocytes by insulin like and glitazone activity	Louis *et al.*,(2006)
30	*Withania somnifera* Dunal.	Hypoglycemic and	Adallu and

		antihyperglycemic effect.	Radhika (2000)
31	*Cajanus cajan* (L) Millsp.	Increases insulin secretion by pancreatic β cells	Ezike*et al.*,(2010)
32	*Citrullus colocynthis* (L.) Schrad.	Stimulates residual pancreatic mechanism,increases peripheral uptake and utilizeation of glucose	Agarwal *et al.*,(2012)
33	*Mangifera indica* L.	Enhances insulin induced glucose uptake through translocation of the glucose transporter	Sangeetha *et al.*,(2010)
34	*Curcuma longa L.*	Regulate two or more pathways (regulation of insulin resistance and β-cell function)	Lekshmi *et al.*,(2012)
35	*Carica. papaya.* L	Regulate β-cell function	Sasidharan *et al.*,(2011)
36	*Laminaria japonica* J.E	Regulate glucose absorption in the guts	Akar *et al.*,(2011)
37	*Zingiber officinale* Roscoe	Regulate two or more pathways (islet cell protection and increased insulin receptor signaling)	Chakraborty *et al.*,(2012)

38	*Cinnamomum zeylanicum* Blume	Regulate insulin resistance	Li *et al.*, (2012)
39	*Trigonella foenum-graecum* L.	Reduces insulin resistance	Uemura *et al.*,(2010)

2.3 Herbal molecules with anti diabetic potentials:

There are more than thousands of plant species being used in the treatment of Diabetes worldwide. Natural phytochemicals isolated from these plant sources may be feasible alternative for treating diabetes. Galegine isolated from Galega officinalis L. showed hypoglycaemic and insulin-sensitizing potential (**Whitters, 2001**). Bellififolin from *Swertia punicea* Hemsl. showed improved insulin sensitivity due to increased expression of key proteins such as insulin receptor (IR), insulin receptor substrate (IRS-1) and phosphatidylinositol-3-kinase (**Tian *et al.*, 2010**).

Rutin, isoquercetin, quercetin, moracin-D, C, N, Norartocarpetin, Chalcomoracin and Euchrenone isolated from *Morus alba* L.showed antidiabetic potential by inhibiting alpha-glucosidase (**Katsube*et al.*,2006**). Curcumin from *Curcuma longa* L. revealed activation of peroxisome proliferator-activated receptor-gamma (PARP-gamma) (**Kuroda *et al.* 2005**). Davidigenin, Sakuranetin, 2,4,-Dihydroxy-4-methoxydihydrochalcone, 4,5-di-O-caffeoylquinic acid, O-caffeonylquinic acid, 6-demethoxycapillarisin from Artemisia dracunculus improved insulin sensitivity by IR signaling and inhibition of aldose reductase (**Wang *et al.*,2011**).

p-Hydroxybenzoic acid, Chlorogenic acid, Vanillic acid, p-Coumaric acid, Ferulic acid, Epicatechin, Catechin, Apigenin, Amentoflavone, Proanthocyanidin, from *Nigella* species inhibits the intestinal absorption of glucose (**Meddah *et al.*, 2009).** Caffeic acid and p-Coumaric acid from *Ocimum sanctum* L. showed alpha-amylase inhibition activity (**Wongsa*et al.* 2012**). Salacinol, Kotalanol, De-O-sulfated salacinol, De-O-sulfated Kotalanol, Ponkolanol and Salaprinol from *Salacia reticulata* Wight. exhibited anti-diabetic effect through inhibition of alpha-glucosidase (**Muraoka *et al.* 2008).**

Piperine, pipernonaline and dehydropipernonaline from *Piper retrofractum* Vahl. revealed antidiabetic effect by activating of proteins from the peroxisome proliferator-activated receptors (**Kimm *et al.*, 2011**). Proanthocyanidin from *Pinus pinaster* Aiton. had alpha glucosidase inhibitory effect (**Bedekar *et al.*, 2010**), Petunidin and Myricetin from *Vitis vinifera* L. possess anti-glycation activity (**Gharib*et al.*, 2013**). Kaempferol-3, neohesperidoside isolated from leaves of *Bauhinia forficata* Link. exhibited significant hypoglycemic effect (**De Sousa *et al.*,2004**). Isorhamnetin from *Hippophae rhamnoides* L. showed anti-diabetic activity by inhibiting glyconeogenesis (**Cao *et al.*, 2003**). Phytoestrogens from *Trigonella foenum-graecum* L. inhibited the diffusion or transport of glucose independent of hormonal mechanism (**Puri, 1999**).

2.4 Siddha System of Medicine

Siddha system of medicine is one of the ancient system developed along the banks of Tamirabarani in the southern peninsular India. About 8,000 herbal remedies have been codified in Siddha literature for treating various diseases. The Agathiyargunavagadam (5000 BC) has recorded 67 medicinal plants, Pathnenkilkanukkunoolgal such as Sirupanjamoolam, Elathy mentioned 81 species, *Atharvaveda* (4500-2500 BC) narrates 290 species, Patharthagunavilakkam and Ayurvedic literature such as *Sushrut Samhita* (200 BC) have described properties and uses of nearly 1100 and 1270 species. These drugs are still used in the classical formulations of the Siddha system of medicine. Unfortunately, much of the ancient knowledge and many valuable plants are lost at an alarming rate. Number of herbs have been long known to possess hypoglycaemic action in both experimental animals and humans. Siddha and Ayurveda, are pioneering systems to give a detailed description on the clinical features and management of *Madhumeha* (Diabetes Mellitus). Efforts are being made to establish their efficacy in controlling diabetes and its various complications that often result in increased morbidity and mortality.

2.5 Siddha formulations used in the management of diabetes

Aavaraiyathichurnam has very good hypoglycaemic effects through enhancing the peripheral utilization of glucose, normalizing the hepatic glycolysis and gluconeogenesis, which were proved by clinical studies (**Anbu and Velpandian, 2012**). Efficacy of *Arugankattai paste* on diabetic neuropathy symptoms like burning sensation was notified by Rajeev and Sewwandi (2013).

Sadagopan *et al.*, (2014) suggested *Madumega Choornam* as beneficial for the treatment of type 2 diabetes. **Kabilan *et al.*, (2013)** studied effect of *Madumega karpam* in the glucose level in alloxan induced albino mice. **Anbu *et al.*, (2012)** confirmed the use of *Palingu Abraga Parpam* for the treatment of Type II diabetes mellitus in a 3 months clinical study.

Yoganandam *et al.*, (2014) reviewed the Siddha kudineer formulation "*AavaraiKudineer*" and found it to be suitable for treating diabetes. *Neerizhivu choornam*, which consists of seven herbal ingredients, was scientifically proved to control the blood sugar level **(Manavalanand and Gopal, 2014). Sathish*et al.*, (2012)** revealed α-glucosidase inhibitory effect of Siddha formulation *Abraga chendhooram*. As many siddha formulations are proven scientifically for their anti diabetic action, in the present work a common siddha formulation on ***Nilavembu Kudineer*** is selected and studied from standardization and validation point of view as no attempts were made on its anti diabetic potentials.

2.6 Literature review on therapeutic potentials of *Nilavembu Kudineer*

In the management of diabetes **Saravana *et al.*, (2015)** focused on statistical optimization of ***Nilavembu Kudineer*** using Response Surface Methodology (RSM) and its antibacterial activity. The combined interactive effect of different variables on ***Nilavembu Kudineer*** production was studied by RSM. The efficiency of the antibacterial compound was tested against clinical isolate such as *Salmonella typhi*. The variables are *Andrographis paniculata* (Burm.f.) Wall., *Piper nigrum* L., *Plectranthus vettiveroides* (Jacob), *Zingiber officinale* Roscoe, *Santalum album* L. *Cyperus rotundus* L, *Hedyotis corymbosa* L, *Trichosanthes cucumerina* L, *and Vetiveria zizanioides* (L). This study suggested that RSM

mediated optimization can be a good method for the enhancement of antimicrobial activity of ***Nilavembu Kudineer***.

Ali *et al.*, (2013) attempted to develop a method to determine the chemical fingerprint of andrographolide present in *Andrographis paniculata* (Burm.f.) Wall., (AP) (Acanthaceae) and ***Nilavembu Kudineer*** churnam (NVK), a siddha poly herbal formulation. The analysis showed that andrographolide concentration was 2.68% in AP and 0.82% in NVK in the 50% methanol extract. As there is no scientific evidence for the anti diabetic activity of ***Nilavembu Kundineer*** in the present dissertation both pre clinical and clinical attempts were made to establish the anti diabetic efficacy of the selected Siddha formulation.

2.7Review on anti diabetic efficacy of ingredients of *Nilavembu Kudineer*

Ingredients of ***Nilavembu Kudineer*** were also assessed individually for their anti diabetic potential. Polyphenol extracts of Ginger (*Zingiber officinale* Roscoe) (Kazeem *et al* 2015) were studied for their ameliorating effects on pancreas and in altering renal derangements in streptozotocin-induced diabetic rats similarly *Piper nigrum* L. was also evaluated for its hypoglycemic potentials **(Onyesife *et al*.,2014).**

Mollugo cerviana L. species were also evaluated for their anti diabetic activity **(Gopalakrishnan *et al* 2010 and R. Valarmathi *et al* 2015)**

Similarly *Vetiveria zizanioides* L. **(Karan *etal* 2013)** *Andrographis paniculata,* **(Premanath *et al* 2015)** *Plectranthus vettiveroides,* **(Gopalakrishnan *et al* 2014)** *Santalum album* L, **(Kulkarni *et al* 2012)** *Trichosanthes cucumerina* L, **(Kirana *et al* 2008)** and *Cyperus rotundus* L **(Raut *et al* 2006)** were also evaluated scientifically for their anti diabetic potentials.

Chapter III

AIM AND OBJECTIVES

3.1 Aim

- To establish safety and anti- diabetic efficacy of Siddha formulation ***Nilavembu Kudineer*** (NVK) and contribute to suffering diabetic population.

3.2 Objectives

- To determine botanical and chemical standards for the selected formulations
- To perform the Acute oral toxicity study of ***Nilavembu Kudineer*** in albino Wistar rats to establish the safety.
- To evaluate anti- diabetic activity of ***Nilavembu Kudineer*** in Streptozotocin induced diabetic rats so as to provide scientific evidences in support of anti diabetic claim of the selected Siddha formulation (NVK).
- To assess Type 2 diabetes mellitus in human subjects using Clinical Siddha Diagnostic and Morden parameters.
- To assess the clinical efficacy of ***Nilavembu Kudineer*** in proven Type 2 diabetes mellitus human subjects.

CHAPTER IV

Work Plan

4.1 Standardization studies

- Identification & authentication of ingredient
- Preparation of the formulation
- Botanical and chemical standardization studies

4.2 Pre-clinical studies

- IAEC Clearance at SASTRA
- Acute Toxicity and Anti Diabetic activity
- Histopathological Evaluation studies
- Data analysis

4.3 Clinical studies

- Ethical Clearance
- Permission from Study centre NIS
- Informed Consent forms collection
- Pilot study
- Clinical assessment
- Data analysis and compilation of all research work

CHAPTER V

Materials and Methods

5 .1.1 Materials and Methods

The formulation ***Nilavembu Kudineer*** (NVK) was selected from the Siddha classical text Siddha vadithyathiratu Various publications depict that each ingredient of ***Nilavembu Kudineer*** is anti- diabetic and is also proved scientifically for their anti diabetic properties. In the present work synergistic anti diabetic efficacy of the ***Nilavembu Kudineer*** is evaluated through pre clinical and clinical studies. Standards as per the Siddha pharmacopoeia is also determined for this formulation to assure the quality of the medicine.

5.1.2 Drug collection, identification and authentication

Selected Siddha drug ***Nilavembu Kudineer*** was purchased from authorized IMCOPS dealer at Trichy. This drug was manufactured by The Indian Medical Practioners Co-operative Pharmacy and Stores Ltd, Thiruvanmiyur, Chennai. The commercially procured formulation was subjected to various studies for evaluating the anti-diabetic efficacy and for the determination of standards in par with modern medicine which is essential for the international recognition and global acceptance of this anti-diabetic Siddha drug.

Preparation of the study drug

The study drug was prepared as per standard protocol following the guidelines of the Drugs and Cosmetics act 1947.

Table:4 Ingredients

S. No.	Ingredients	Botanical name	Part used	Quantity
1	Nilavembu	*Andrographis paniculata* Burm. f	leaves	8.75gms
2	Vilamichamver	*Plectranthus vettiveroides* Jacob	Root	8.75gms
3	Santhanam	*Santalum album* L.	Wood	8.75gms
4	Peipudal	*Trichosanthes cucumerina* L.	Whole plant	8.75gms
5	Koraikizhangu	*Cyperus rotundus* L.	Root	8.75gms
6	Chukku	*Zingiber officinale* Roscoe	Root	8.75gms
7	Milagu	*Piper nigrum* L.	Fruit seed	8.75gms
8	Parpatakam	*Mollugo cerviana* L. Ser	Whole plant	8.75gms
9	Vettiver	*Vetiveria zizanioides* L.	Root	8.75gms

5.2 Method of preparation

The above purified ingredients are dried and made of aqueous decoction.

Dosage 30- 60ml once a day.(Oral)

Duration 45 days

5.2.1 Preparation of aqueous extract

Plant materials were wasted thoroughly with water and cut into small pieces, shade dried coarsely powdered and extracted with water. 200 g of plant material was soaked in water (2 L) and heated at 80°C for 1 h. The crude extract was filtered using Whatman paper and evaporated *in vacuo* at 60°C using a rotary evaporator.

Ingredients of *Nilavembu kudineer*

(**A).** ***Andrographis paniculata*** **Burm. f. Leaves & stem (Nilavembu)**

(B). ***Plectranthus vettiveroides*** **Jacob. Root (Vilamichamver)**

(C). *Santalum album* L. Wood (santhanam)

(**D). *Trichosanthes cucumerina* L. Whole plant (Peipudal)**

(E). *Cyperus rotundus* L. Rhizome (Koraikizhangu)

(F). *Zingiber officinale* Roscoe Rhizome (Chukku)

(G). ***Piper nigrum*** **L. Fruit (Milagu)**

(H). *Mollugo cerviana* (L.) Ser. Whole plant (Parpatakam)

(I). *Vetiveria zizanioides* L. Root (Vettiver)

(J). Nilavembu powder (Raw material for kudineer)

(K). *Nilavembu kudineer*

Color: Greenish brown
Taste: Bitter
Odor: Slightly aromatic

5.3 Botanical Characterization

5.3.1Organoleptic evaluation

Organoleptic evaluation is carried out as per Ayurvedic Pharmacopoeial procedures (2004). Standards were evaluated and tabulated.

5.3.2 Microscopic studies

Powder Microscopy

Powder microscopy helps to visualise crystals, trichomes, leaf epidermal cells and parenchyma cells.

A pinch of powdered material was placed on a microscopic slide. A small amount of chloral hydrate and few drops water added. The slide was warmed over a water bath for few minutes. The samples treated with chloral hydrate mounted with 30% glycerol and observed under microscope.

Slides prepared using the test drug were observed under microscope (Zeiss, Scope A-1, Germany) and microscopic images were captured using CC camera (JENOPTIK, Germany) and ProRes C5 software.

5.3.4Histochemical Studies

Test for Lignin

A pinch of the powdered plant material was placed on a microscopic slide and stained with 1% solution of phloroglucinol in ethanol for 1- 2 mins. The phloroglucinol drained and few drops of conc. HCl added. The excess acid also drained off and few drops of 30% glycerol added and then treated powder was mounted and observed under microscope. Pink to cherry red in colour observed indicates the presence of lignin in the plant.

Test for Starch

A pinch of the powdered plant material was placed on a microscopic slide and stained with iodine in potassium iodide solution. Cover glass placed over it and observed under the microscope. Starch grains will stain dark blue to dark purple in colour**.**

Test for fats and fatty oil

A pinch of powdered material placed on a microscopic slide and stained with 2-3 drops sudan red and allowed to stand for few minutes and observed under microscope. The development of orange red to red in colour indicates the presence of fats, fatty oils, volatile oil and resins. The slides were irrigated with ethanol (750/L) and heated gently. Colour obtained due to the presence of volatile oils and resins get dissolved in ethanol. The presence of fats and fattly oils were confirmed by the intact orange red to red colour.

Test for calcium Carbonate crystals

A small quantity of powdered plant material placed on a microscopic slide and treated with few drops of acetic acid (60g/L) or hydrochloric acid (70g/L) solution. Dissolving of crystals with effervescence indicates the presence of calcium carbonate crystal.

Test for calcium oxalate crystals

A small quantity of powdered plant material was placed on a microscopic slide and treated with few drops of acetic acid (60g/L) or hydrochloric acid (70g/L) solution.
Crystals of calcium oxalate are insoluble in acetic acid solution, but soluble in hydrochloric acid solution without effervescence.

5.4 Chemical standardization studies

5.4.1Tests for identity, purity and strength (Ayurvedic Pharmacopoeia of India, 2004)

5.4.2 Determination of foreign matter

Drug sample (100 g) was weighed and spread out in a thin layer (Ayurvedic Pharmacopoeia of India, 2004). The foreign matter was detected by inspection with the unaided eye or by the use of a lens (6 x). It was separated and weighed and the percentage was calculated.

5.4.3 Determination of total ash

About 2 g of the powdered drug was weighed in a silica dish at a temperature not exceeding 450ºC until free from carbon, cool and weigh (Ayurvedic Pharmacopoeia of India, 2004). The charred mass was extracted with hot water, the residue was collected in an ashless

filter paper, and then the residue was incinerated. The filtrate was evaporated to dryness, and ignited at a temperature not exceeding 450ºC. The percentage of ash was calculated with reference to the air-dried drug.

5.4.4 Determination of acid insoluble ash (Ayurvedic Pharmacopoeia of India, 2004)

The ash obtained from total ash was boiled for 5 minutes with 25 ml of dilute hydrochloric acid. The insoluble matter was collected in an ashless filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash was calculated with reference to the air dried drug

5.4.5 Preliminary phytochemical screening of plant extracts

Suitable quantity of ***Nilavembu Kudineer*** powder were subsequently extracted and phytochemical test were conducted as per methods of Harbone (1973).

5.5 Estimation of alkaloids (Harbone, 1973)

About 2 grams of the powdered sample was mixed with l g of calcium hydroxide and 5 ml of water into a smooth paste and was set aside for five minutes. It was then evaporated to dryness in a porcelain dish on a water bath. To this 200 ml of chloroform was added, mixed well and refluxed for half an hour on a water bath. Then it was filtered and the chloroform was evaporated. To this 5 ml of dilute hydrochloric acid was added followed by 2 ml of each of the following reagents.

(i) Mayer’s reagent
(ii) Dragendorff’s reagent
(iii) Hager’s reagent
(iv) Wagner’s reagent

Observations were recorded.

5.5.1 Estimation of mucilage

10 ml of concentrated aqueous extract was taken and evaporated to dryness. The dried mass was dissolved in water. The dissolved mass is transferred into 50ml of absolute alcohol drop wise. The precipitate formed was allowed to settle for one hour and was filtered through tarred sintered crucible. The precipitate was weighed and amount was calculated.

5.5.2 Estimation of Carbohydrates

Weigh 100mg of sample in a boiling tube. Hydrolyze by keeping it in a boiling water bath for 3 h with 5ml of 2.5N HCl and cool to room temperature. Make the volume to 100ml and centrifuge. Collect the supernatant and take 0.5ml and 1ml for analysis. Prepare the standards by taking 0.2, 0.4, 0.6, 0.8 and 1.0ml of the working standard. Make up the volume to 1ml in all the tubes including the sample tubes by adding distilled water. Then add 4ml of anthrone reagent. Heat for eight minutes in a boiling water bath. Cool rapidly and read the green to dark green colour at 630nm.

Calculation

Sample absorbance x Standard weight x Sample dilution x100

Standard absorbance x Standard dilution x Sample weight

5.6 HPTLC Analysis

5.6.1Chromatographic conditions

The experiment was performed on a precoated silica gel 60 F_{254} (0.2 mm thickness) HPTLC plates of E.Merck ,Germany (10x10cm). Samples were applied to the plates as 7mm bands, 15mm apart from the edges of the plate, with a Camag Linomat5 sample applicator. The plates were developed to a distance of 80mm, in a Camag twin trough glass chamber, using a mobile phase chloroform: Methanol (7:1). The saturation time was kept for 30min. After development, plates were dried in a hot-air oven, viewed in a Camag UV chamber. The chromatograms were scanned using Camag TLC Scanner3. The R_f values and fingerprint data were recorded by WINCATS software version 1.3.4.

5.6.2Sample Preparation

Refluxed 5g of powdered drug successively with Petroleum ether, chloroform and methanol. Filtered and concentrated the methanol extract to dryness. Dissolve 10mg of residue in 1ml of methanol.

Standard Preparation

1.3mg of andrographolide was dissolved in 1ml of methanol.

5.7 GC-MS Analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system interfaced to a Mass Spectrometer (GC-MS) instrument employing the following conditions: column Elite-5m fused silica capillary column (30 x 0.25 mm ID x 0.25µm film thickness, composed of crossbond 5% phenyl 95% Dimethyl polysiloxane), Helium (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 1.0 µl was employed (split ratio of 10:1) injector temperature 290 ºC; ion-source temperature 160ºC. The oven temperature was programmed from 50ºC, with an increase of 8 ºC/min to 220ºC hold for 5min, then 8ºC /min to 280ºC hold for 10 min. Mass spectra were taken at 70eV at a scan interval of 0.2 seconds and fragments were scanned from 40 to 600 Da.

Instrument Details:

Make : PerkinElmer Clarus 500

Software : Turbomass ver5.2.0

Column Type : Capillary Column Elite-5 (Crossbond 5%Phenyl 95% dimethylpolysiloxane)

Column length: 30m

Column id : 250µm

GC Conditions:

Oven	Rate	Temp	Hold
Initial	---	60	0.00
1	6.0	150	2.00
2	4.0	280	5.00

Injector temp. : 280ºC

Carrier gas : Helium @ flow rate 1ml/min

Split ratio : 1:10

MS Conditions:

Mass Range : 40-600amu

Type of Ionization : Electron Ionization (EI)

Electron energy : 70ev

Transfer line and source temperature: 200ºC, 160ºC

Sample injected : 1.0µL

Identification of components

Interpretation on mass spectrum GC-MS was done using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the separated components was compared with the spectrum of NIST library database. The identity of the spectra above 95% was needed for the identification of components.

5.8 FTIR Analysis

PerkinElmer spectrometer FT-IR Spectrum one in the range of 4000–400cm^{-1}at a resolution of 4$cm^{-1}$was used in this analysis that helps to determine the chemical functional groups present in the sample which is based on the absorbance of various characteristic frequencies by the different functional groups present in the given sample.

A solid sample about 10 µg was ground well into KBr matrix and analyzed for determining various functional groups. The sample was mixed with KBr procured from Merk chemicals. Thin sample pellet was prepared by pressing with the Hydraulic Pellet Press and subjected to FT-IR analysis.

5.9 Heavy Metal Analysis (AAS)

Atomic Absorption Spectroscopy (AAS) is used for detecting presence of heavy metals. Atomic absorption is the process that occurs when a ground state atom absorbs energy in the form of light to a specific wavelength and was elevated to an excited state. The amount of light energy absorbed at this wavelength increased as the number of atoms of the selected element in the light path increases. The relationship between the amount of light absorbed and the concentration of analytes present in known standards could be used to determine unknown sample concentration by measuring the amount of light, they absorbed.

The absorption of light was proportional to the concentration of free atoms in the flame. It was given by Lambart-beer law.

Absorbance = log 10 I_0/I_t = k.c.l

Where,

I_o = intensity of incident radiation emitted by the light source.

I_t = intensity of transmitted radiation.

c = concentration of sample (free atoms).

k = constant **l** = path length

5.9.1 Sample Collection

The samples were cleaned and dried under shade. Then, the samples were dried in an oven at 40°-50° C till a constant weight was obtained. The dried samples were then, ground and powdered with agate pestle and mortar. Samples were labeled and stored in pre-cleaned polyethylene bottles for further analysis.

5.9.2Reagents and Apparatus

All the reagents such as HNO_3 and H_2O_2 used in the study were purchased from MERCK (Analytical Grade). De-ionized water was used for all analytical work and all the glassware's, polyethylene bottles, pipette tips and others were washed with 1 % HCl, rinsed with de-ionized water before preparing standards, reagents and samples.

5.9.3Sample Preparation (Ash Method)

The collected medicinal plant parts were cleaned and dried under shade. The dried part was then, ground to fine powder, which was used for drying to ashing. Necessary precautions were taken at every step to avoid metallic contamination in any form. Pre-cleaned silica crucibles were kept in muffle furnace maintained at 600° C. Until the weight of the crucible reached a constant level, the crucible was kept in the furnace. Powdered plant material (5 gm) were taken in the silica crucible and maintained in a muffle furnace at 600° C for 6 hrs. The crucible were then, taken out and cooled at room temperature by keeping it in a desiccator and then, the ash values were measured. Then, the ash was dissolved in 100 ml of

5 % HCL. The dissolved ash solutions were filtered through Whatman filter paper No.40 and are stored in tightly capped plastic bottles. The prepared solutions were directly subjected to flame photometry and AAS for the estimation of various elemental concentrations.

5.9.4Digestion of Samples

A Multiwave 3000 micro oven system (from Anton paar, USA) with 16 positions for Teflon vessels with capping was being used here. The digestion vessels were provided with a controlled pressure, temperature and release valve. Before use, all Teflon vessels were soaked with 10 % HNO_3. The system was initially programmed by giving gradual rise of 20 %, 40 %, and 50 % power for 5, 15 and 20 minutes, respectively for the due warming up. The powder samples were being used without any further treatment for sample preparation. 0.2 gm of sample was weighed into the Teflon vessels followed by digestion mixture of HNO_3 and H_2O_2 in the ratio of 3:1. According to the nature of samples, the ratio was being applied. The resulting solution after microwave digestion was filtered through Whatman 40 filter paper (if necessary) and diluted to 50 ml with de-ionized water. A sample blank containing only acid mixture was prepared at the same time. The method of standard addition was generally adapted to calibrate the instrument before going for the observation of the samples.

5.9.5Determination of Metals

All the atomic measurements were carried out with PerkinElmer model 400/HGA900/AS800 coupled with Mercury Hydride System-15 (MHS-15) and Flame Photometer. The Electrodeless Discharge Lamp (EDL) for Cd, Pb, Hg and As analyses were used as a light source to provide specific wavelength of the elements to be determined High purity (99.999 %) Acetylene and Nitrous oxide were used to provide constant thermal energy for atomization process and Argon gas used for carrier gas purging purposes for Graphite furnace.

Calibration of Instruments

More than three working standard solution of elements to be determined were prepared, covering the concentration range as recommended by the manufacturer of the instrument for the elements to be determined. Before the analysis of samples, the instruments were calibrated with prepared working standard solution. The calibration curve was obtained

for concentration vs. absorbance and data obtained was statistically analyzed. Calibration of the instrument was repeated periodically during operations and blanks were carried with each set of 10 samples or aspirated any one of the prepared working standard for every 10 samples to check the instrument drift and to validate analytical procedures and performance. Regent blank reading would be taken and necessary correction would be made during the calculation of concentration of various elements. Standard Certified Reference of National Institute of Standard and Technology (NIST) was used for day-to-day, and for the evaluation of methods of analysis or test and also for long-term quality assurance of measurements. A reagent blank reading was taken and necessary corrections were made during the calculation of concentration of various elements (**Eng-Shi et al., 2000 and Sahito et al., 2001**).

5.10 Microbial Analysis

Materials containing microorganism were cultured. Each viable organism developed into a colony. Hence, the number of colonies appearing on a plate represented the number of living organisms present in the given sample.

Procedure

Plant material samples were obtained and thoroughly mixed to make composite sample for microbial analysis. 10 gm of appropriate plant material was weighed and 100 ml of sterile distilled water in a sterilized conical flask were placed for serial dilution. The flasks were kept in a mechanical shaker for five minutes to obtain uniform suspension of microorganisms. The dilution level was 1-10 or 10^{-1}. From this, 1 ml of dilution from 10^{-1} sample was taken out and if transferred into 9 ml, this was 10^{-2} dilution. The procedure was repeated up to 10^{-6} dilution. Transfer 1ml of serial dilution from10^{-1} to 10^{-6} into sterilized petri-plates for enumerating bacteria, fungi and severe pathogens such as *Salmonella, Shigella* and *E.coli*. Two replications are maintained to each dilution, in each group of microorganisms. The medium such as Nutrient Agar (NA), Potato Dextrose Agar (PDA), Salmonella Shigella Agar (SS) and Eosin Methylene Blue Agar (EMB) were added to the sterilized petri-plate with one mL sample, and rotating the plate clockwise and anti clockwise to get an uniform distribution of microbial cells.

The medium was allowed to set and the plates were incubated in inverted position at 37^0C for about 1-2 days for bacteria and 3-5 days for fungi. The colonies were counted on the plates with the aid of colony counter. The numbers of colonies were observed from both the plates, which were kept for replication. The total numbers of population were enumerated individually for fungi and bacteria by taking the average of the two dilutions employed and expressed in 1 gm of plant material.

5.11 X-ray Fluorescence spectrometer (XRF)

Method

XRF is a technique that measures the concentration of elements present in a given sample through detection of secondary electrons.

Equipment

Instrument: XRF Bruker S8 Tiger

X-ray fluorescence spectrometry: Two grams of boric acid were filled in aluminium cups and then 1 gram of each sample was evenly spread over it. The aluminium cups were pelletized using a hydraulic press at 25 tons to obtain pellets. Samples were analyzed using XRF spectrometer (Bruker S8 Tiger) equipped with a 4KW, Rh anode X-ray tube.

5.12 In vitro studies

***In vitro* cytotoxicity studies**

Evaluation of cytotoxicity of aqueous extract of *Nilavembu Kudineer*

Methodology

Extract preparation

The Siddha drug ***Nilavembu Kudineer*** was purchased from authorized dealer at Trichy. This drug was manufactured by The Indian Medical Practioners Co-operative Pharmacy and Stores Ltd, Thiruvanmiyur, Chennai. This drug was composed of Seemai Nilavembu (*Andrographis paniculata* Burm. f), vettiver (*Vetiveria zizanioides* L.), vilamichamver (*Plectranthus vettiveroides* Jacob), santhanam (*Santalum album* L.), peipudal (*Trichosanthes cucumerina* L.), koraikizhangu (*Cyperus rotundus* L.), chukka (*Zingiber officinale* Roscoe), milagu (*Piper nigrum* L.) and parpatakam (*Mollugo cerviana* L. Ser). The

aqueous extract was prepared by taking 25 g of ***Nilavembu Kudineer*** raw material in 500 ml of distilled water and boiled at 90°C until the final volume reaches to 100 ml. Then the content was filtered and the filtrate was frozen and then lyophilized. The extract yield was found to be 1.32 g/100 g drug. The extract was re-suspended in water at 10 mg/ml ratio and used for the cytotoxicity experiment.

Evaluation of cytotoxicity

The 3T3-L1 pre-adipocytes were obtained from the National Center for Cell Science (NCCS), Pune and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and penicillin (100 U/mL)-streptomycin (100 µg/mL) in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. After arriving 60% of confluency, the cells were trypsinized and dispersed in a 96 well plate with a cell count of 9000 cells per well and incubated for 24 h. Then the aqueous extract was added at different concentrations (10, 5, 2.5, 1.25 and 0.625 mg/ml) and then again incubated for 24 h. The cell group grown in medium without plant extract was considered as control. At the end, the medium was discarded, cells are washed with PBS and then MTT reagent (20 microliter) was added in each well and incubated for 6 h at 37°C in a water bath. Then 150 µl of acidic isopropanol was added and shaken for 30 min on a plate shaker under dark. The absorbance was measured at 540 nm in a micro-plate reader and the cell viability was calculated and expressed in percentage basis.

5.13 Pre- Clinical Studies

5.13.1 Acute Oral Toxicity Study

Acute Oral Toxicity Study of ***Nilavembu Kudineer*** (NVK) in rats was performed as per Organization for Economic Cooperation and Development (OECD) Test Guidelines - 425 for the conduct of Acute Oral Toxicity (Up and Down Procedure) with slight modifications was followed (OECD, 2008). All procedures involving laboratory animal use were in accordance with the Institute Animal Ethics Committee (IAEC) regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the approval number is **357/SASTRA/IAEC/RPP**.

5.13.2Selection of animals

Healthy female Wistar Rats (*Rattus norvegicus*) between 8 and 12 weeks old and individual body weights falling within ± 20 % of the mean initial body weight were selected for the present study. The female rats used for the present study were nulliparous and non-pregnant.

5.13.3Identification of Animals

Tags marked with animal number, group number and dose level were attached to the respective cages. Each animal was identified by unique identification number by ear tagging.

Table: 5 Identification of Animals

Animal ID	Sex
6022	Female
6023	Female
6024	Female
6025	Female
6026	Female

Acclimatization

Animals were acclimatized to the study environment for a week prior to drug treatment. During the acclimatization period, all the animals were observed for clinical signs once a day. At the time of receipt of animals and completion of the acclimatization period, the body weights were measured using the automatic animal balance (Sartorius, Germany).

Animal Husbandry

Animal House Condition

Temperature of the test room was maintained between 22±3°C and relative humidity between 50 to 70% during the experimental period. The experimental room was provided with a 12h light and 12h dark lighting condition using an automatic timer.

Housing

Standard polypropylene rat cages with stainless steel top grill was used to house the animals. The cages were autoclaved. Sieved and sterilized paddy husk was used as the bedding material. Animals were housed individually.

Sanitation

Bedding material , cages, grills and water bottles were changed weekly twice.

Animal Welfare and Regulatory Compliance

The experiment was conducted at the Central Animal Facility registered (No.817/04/ac/CPCSEA dated11.03.99) for Breeding and Experiments of Animals by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Forest and Environment, Govt. of India.

The study was conducted after the approval by the Institutional Animal Ethical Committee, SASTRA University (IAEC Approval Number: 357/SASTRA/IAEC/RPP).

Diet and Water

Standard rodent pellet feed supplied by M/s. ATNT Laboratories, Mumbai, India and Reverse Osmosis (RO) water were provided to the animals *ad libitum*.

Preparation of Test Substance

The test substance, ***Nilavembu Kudineer*** was dissolved in distilled water. The test substance was prepared freshly before dosing and the dose was calculated as per the body weight of individual animals.

5.13.4Experimental Plan

Healthy Female Albino Wistar rats were selected for the studies and administered with a single oral dose of aqueous extract of ***Nilavembu Kudineer*** at the dose of 2000 mg/kg,

b.wt by oral route dissolved in 1 mL of distilled water used as the vehicle. Each animal was observed for every 15 min in the first 4 h after dosing, then every 30 min for the successive 6 h and then daily for the successive 48 h for the short-term outcome and the remaining 14 days for the long-term possible lethal outcome which in this case was death. The animals were observed for signs of convulsions, tremors, circling, depression, excitement and mortality. The surviving animals were sacrificed under CO_2 inhalation euthanasia, autopsied and examined macroscopically for any pathological changes.

Mortality

All animals were observed twice everyday for mortality for 14 days.

Body Weight

Body weight of each animal was recorded just prior to the test substance treatment on Day 0, Day 7 and Day 14 using electronic Animal weighing balance (Sartorius AG, Germany).

Feed Intake

Feed intake for individual animals was recorded daily for the entire study period.

Table:6 Dosing schedule for individual animals

Animal ID	Dose (mg/kg)	Observations
6022	2000	Survived
6023	2000	Survived
6024	2000	Survived
6025	2000	Survived
6026	2000	Survived

OBSERVATIONS

Observation Period: 14 days

5.13.5Clinical signs observations

During the observation period, the drug treated animals were observed for its clinical signs of toxicity just before the administration, at 30 minutes, 1, 2, 3, 4 and 8 hours after the administration and at least once a day from day 1 till 14. Clinical signs including mortality, morbidity, general appearance (skin and fur, eye and mucous membrane, nervous system etc.) and behavior were recorded. The clinical observations such as faeces, colour and consistency, clear ocular discharge, hyper activity, skin and fur examination, tremor and convulsion, pilo erection, change in gait, repetitive circling and excessive grooming were observed in all test drug treated experimental animals.

Gross Pathology

All animals subjected to necropsy were showed no gross pathological changes.

5.13.6 ANTI-DIABETIC SCREENING STUDIES

Anti-diabetic screening of *Nilavembu Kudineer* against Streptozotocin (STZ)-Induced Diabetics in Rats (Syed A Hassan et al., 2012)

Table: 7 Experimental Design

Groups	Descriptions	No. of Animals	Treatments
I	Normal control	6	Distilled water
II	Diseased control	6	Streptozotocin (STZ, 65 mg/kg, i.p)
III	Standard	6	STZ (65 mg/kg, i.p)+ Metformin (100 mg/kg, p.o)
IV	NK - Low dose	6	STZ (65 mg/kg, i.p)+ *Nilavembu Kudineer* (100 mg/kg, p.o)
V	NK - Medium dose	6	STZ (65 mg/kg, i.p)+ *Nilavembu Kudineer* (200 mg/kg, p.o)
VI	NK - High dose	6	STZ (65 mg/kg, i.p)+ *Nilavembu Kudineer* (400 mg/kg, p.o)

NVK- ***Nilavembu Kudineer***, *p.o.*-per oral, STZ-Streptozotocin, i,p-intraperitoneal

5.13.7Experimental procedure

The diabetes mellitus was induced by a single intraperitoneal injection of Streptozotocin (STZ, 65 mg/kg) dissolved in 0.1M citrate buffer (pH 4.5) in fasted male Wistar rats. The control group rats were treated with distilled water. The Diabetes was confirmed at 48 h after STZ injection by measuring the glucose concentrations of peripheral blood, obtained from the tail vein using glucometer (ASPEN AP +PLUS). Rats with blood glucose levels of 250 mg/dl or higher were considered to be diabetic and were grouped into five groups each containing eight rats (Akbarzadeh et al., 2007). The diabetic induced rats were treated with standard/vehicle/test drug orally as per the study protocol (Table7) for 4 weeks. The change in body weights were measured weekly. During the last week of drug treatment, experimental rats were housed in metabolic cages and the changes in feed intake, water intake and urine output were measured and calculated. At the end of the experiment, all the rats were fasted overnight and blood samples were collected by retro orbital puncture under anesthesia. The blood samples were collected in clot activator tubes and allowed to clot for 10 minutes, centrifuged at 2500 rpm for 15 minutes to obtain serum. The serum layer was collected in properly labeled, clean and micro-centrifuge tubes and analyzed immediately for biochemical parameters such as glucose, total protein, Albumin, Creatinine, Urea, total cholesterol and triglycerides levels.

5.14 Clinical Studies

Acute oral toxicity studies for ***Nilavembu Kudineer*** was performed as per OECD – 425 guidelines from the toxicity results it was observed that there was no significant changes in weekly bodyweight and also daily behavior observation. It is concluded from the study that the drug is safe upto 2000 mg/kg when administered orally. After ascertaining the anti diabetic efficacy of ***Nilavembu Kudineer*** in pre clinical studies conducted using STZ induced diabetic rat models and ascertaining nontoxic nature of the test drug ***Nilavembu Kudineer,*** a pilot scale clinical study was under taken.

5.14.1 Ethical clearance

Institutional Ethical Committee (IEC) of National Institute of Siddha, Tambaram Santorium, Chennai, approved the protocol before the initiation of the trial of ***Nilavembu***

Kudineer in diabetic subjects. This clinical trial is registered in Clinical Trial Registry of India (CTRI) New Delhi.

Clinical study was conducted as per the guidelines for Good Clinical Practice accepted by AYUSH adapted from the International Conference on Harmonization (ICH). The clinical study was carried out in two phases as pilot study and as main study.

Study design: Open clinical trial.

Sample size: Sample size arrived was 60

Study Centre: Ayothidoss Pandithar Hospital, National Institute of Siddha, Tambaram, Chennai- 47.

Treatment period: 90 days and follow-up period for 1 month.

Study population: Diabetic Patients in the age group of 30 to 60 years, both male and female

5.14.2Inclusion criteria

1. Both the sexes.
2. Age group of 30 to 60 years with history of NIDDM for less than 5 years were included.
3. Newly diagnosed of NIDDM patients from Out Patient Department, Ayothidoss Pandithar Hospital, were included in this study
4. Patients with or without symptoms like polyuria, polydipsia, polyphagia and weight loss were included in this study
5. Blood glucose levels:

Fasting glucose level	:	≥ 126 mg/dl to	≤ 250 mg/ dl
Post prandial glucose level	:	≥ 200 mg/dl to	≤ 400 mg/ dl
HbA1c	:	6.5 mmol/l to	8 mmol/l

5.14.2Exclusion Criteria

1. Patients below 30 years and above 60 yrs.
2 Insulin dependent Diabetes Mellitus
3. Gestational Diabetes
4. Diabetes patients with complications
5. Known diabetic patients for more than 5yrs were excluded
6. Insulin dependent diabetes mellitus. Gestational diabetes / lactating and pregnant women

Withdrawal criteria

Subjects with severe occurrence of adverse events or signs of severity were excluded from the clinical trial. These patients were withdrawn from the clinical trial studies.

5.14.3Laboratory Diagnostic Parameters

Patients were screened for following Laboratory Diagnostic Parameters.

Hematology: Hb, TC, DC, TRBC, PCV, MCV, PLT, ESR.

Liver function tests: Serum bilirubin, SGOT, SGPT, Serum Alkaline Phosphatese.

Renal function tests: Blood urea, Creatinine, total Protein, Albumin, globulin, uric acid.

Urine examination: Fasting Urine Sugar, Postprandial urine sugar, Albumin, Pus cells, epithelial cells. Deposits, Bile salt, Bile pigments.

Blood: HbA1c.

Lipid profile: cholesterol, HDL, LDL, VLDL, TGL.

Urine examination: ketones, pH of urine, Specific gravity.

5.14.3Siddha Diagnostic Parameters

They were evaluated for following Siddha diagnostic parameters

- Thinai, Theganilai, Noyinmukkutranilai, saptha thadhukkal, migugunam, kuraigunam, Uyirthadhukal migugunam, kuraigunam,
- Envagaithervugal Na, niram, mozhi, vizhi, sparisam, malam, moothiram- Neerkuri, Neikuri, Naadi.

Table: 8 Treatment Regimen

S. No.	Drug Administration Protocol	
1	Study drug	***Nilavembu Kudineer***
2	Route of Administration	Oral administration
3	Dosage of the Medicine	30 -60 ml once
4	Duration of the Treatment	90 days

Patients of age group between 30yrs and 60 yrs of both sexes who visited Out-Patient Department of National Institute of Siddha, Chennai-47 with the symptoms of polyuria, polydipsia, and polyphagia were included in this study. Patients having history of Diabetes Mellitus prevailing less than 5 years were recruited in this study after informing and getting written consent from them that they were willing to take part in the anti diabetic screening of ***Nilavembu Kudineer*** and for evaluating HbA1c, fasting and post prandial blood sugar levels.

The patients who were under the treatment for diabetes were asked to withdraw previous medicines for one day before initialing the study drug clinical trial. The history, symptoms, and signs were recorded in the case sheet. ***Nilavembu Kudineer*** 60 ml was given to selected human subjects for 7days. Before starting the treatment, patients were instructed about dosage, mode of administration, frequency of administration of ***Nilavembu Kudineer***. The patients were advised to follow the diet restrictions, exercise and yoga as stated in the

literature. The patients were asked to come for review once in 7 days and to collect the study medicine. In addition to Fasting and postprandial urine sugar, ketone bodies analysis were done in urine test. Signs and symptoms of hypoglycemic conditions and hyperglycemic conditions were explained to the patients and their relatives to identify the severity of the disease. Patients were informed about the adverse effects i.e. rashes, vomiting diarrhoea, etc. and asked to inform immediately in case of to the Principle Investigator.

No one from study population reported adverse effects. Patients were examined and signs and symptoms were recorded in the case sheet. The blood sugar test for Fasting and Post Prandial were carried out to assess the prognosis of the disease. Complete Blood Count, Renal profile, Liver function test, Lipid profile, Total protein, albumin, Globulin, and uric acid were done on 0 day and repeated on 90^{th} day to assess the prognosis of the disease and efficacy of the study drug.

5.14.4Informed consent

Participants were informed well about the research study, risk and benefits of participation. After getting their voluntary consent they were enrolled for the study.

Pilot study

Initially, a Pilot study was conducted to validate the study protocol.

Main study

After the pilot study, main study was carried out.

Medicine intake chart was provided to mark an entry of intake of the medicine for 90 days. All the patients had the ***Nilavembu Kudineer*** 60 ml once daily regularly without any break.

5.14.5 Assessment of the Siddha parameters

Theganilai (Body Constitution) and Noinilai (Disease condition) were assessed initially. According to the Siddha concept, *Thinai* (Land Tract)of the patients were recorded. Food habits responsible for the occurrence of diabetes, according to the Siddha concept were recorded. Changes in the *Ennvagaithervu* i.e., Na (Tongue), *Niram* (Colour of the Body), *Mozhi* (Speech), *Vizhi* (Vision), *Sparisam* (Palpation), *Malam* (Stools), *Moothiram* (Urine)- *Neerkuri, Neikuri* and *Naadi* were examined and recorded in the case sheet for all the patients. No one from the study participants was reported of adverse effects during the treatment period and follow up period,

On completion of the study period, the patients were followed up for a year. During that period, patients were asked to come for review every 15 days and the study drug was administered throughout the follow up period. HbA_1c test was done before and after treatment.

5.14.6Follow-up visits were needed to

1. Carryout procedures specified in the study protocol, including treatment administration
2. Evaluate the patient's response to treatment
3. Assess patient adherence to the assigned treatment
4. Collect data needed for evaluation of the treatment such as to assess the.

   Rate of occurrence of the outcome(s) of interest

   Patient health care

   Patient convenience considerations

Table:9 Parameters observed to assess the efficacy of the selected drug

S. No	Parameters	0 Day	30th Day	45h Day	60 th Day	90 th Day
1.	Polyphagia	A	A	A	A	A
2.	Polydypsia	A	A	A	A	A
3.	Polyuria	A	A	A	A	A
4.	Loss of weight	A	A	A	A	A
5.	Pain/cramps	A	A	A	A	A

6.	Blood sugar FBS/PPBS	A	A	A	A	A
7.	Haemogram	A	NA	NA	NA	A
8.	Cholesterol	A	NA	NA	NA	A
9.	Triglycerids	A	NA	NA	NA	A
10.	Hb A1c	A	NA	NA	NA	A
11.	LFT	A	NA	NA	NA	A
12.	KFT	A	NA	NA	NA	A

A – Applicable

NA – not applicable

FBS – Fasting blood sugar

LFT – Liver function test

PPBS – Post prandial blood sugar

KFT – Kidney function test

5.14.7Outcome Measures

Primary outcome

Blood glucose and changes of clinical symptoms.

Secondary outcome

Relief from symptoms such as, Poly urea, poly phagia, polydypsia ,itching and numbness, changes in other investigatory parameters were taken into account.

5.14.8Periodical report on the patients

All measurements and parameters were observed regularly. Priority was given for the tests mentioned in the case record form and the reports recorded periodically for ascertaining the efficacy and for observing the statistical significance.

On receiving the results the patients were called for individual counseling and further proceedings. After this process of finishing the initial measurement parameters and screening of general information, the study was initiated with the 0 day as the starting point with a diet

list – Pathiyam as per Siddha system which was given to each subject for adhering to it strictly throughout the study period of 180 days.

5.14.9Data processing

During this study piling up of patients records for a long time is avoided. Organized check was followed on trial progress in the early stages. Data processing for statistical analysis was filled right from very beginning.

In this study on the anti-diabetic efficacy of ***Nilavembu Kudineer***, a collection of observations were recorded of the subjects for 90days, (the study period) and consolidated as a data. They were condensed and presented in the form of a table to improve communication. The table helped the investigator to grasp the overall nature of the data.

5.14.10 Statistical analysis

All the demographic and clinical and investigative data which were entered into the case recording form were exported into an excel sheet. The variables were coded and entered in the data sheet. The cumulative data sheet was electronically maintained to facilitate the data analysis by using the SPSS software.

Chapter - VI

Results

6.1 Botanical studies

Powder microscopy of ***Nilavembu kudineer*** revealed the presence of prismatic, acicular and druses type of calcium oxalate crystals. Unicellular, uniseriate and stellate non glandular trichomes are seen with smooth and warty surface. Lumen is wide and narrow. Uniseriate glandular trichomes are present with and without heads and the cells are filled with cellular contents. Some of the trichomes are seen with degenerated cells. Simple and compound starch grains present with striated margin. Peaked, hilum acentric and fissured. Compound starch grains contains di, tri, tetra or many grains. Macro, brachy and trichosclereids are seen with pitted, striated thick lignified cell wall. Lumen is wide and narrow, branched and simple. Libriform and tracheid fibres are seen with simple and bordered pits and the ends are tapering and blunt, some of the fibres are bifurcated. Testa is made up of sclerenchymatous thick walled pitted cells with branched narrow and simple wide lumen and minute pores are seen in corners where the two cells are joined. The xylem vessels are long and narrow or short and wide tailed with simple and bordered pitted, scalariform, reticulate and spiral thickenings. Epidermal cells are wavy, straight and beaded with druses of calcium oxalate crystals. Some of the cortical cells are thick with lignified walls, containing prismatic calcium oxalate crystals.

Table:10 Cells and cell inclusions of ***Nilavembu Kudineer***

S. No	Cells/ cell inclusions	Size in µm
1	Xylem vessels	140 - 248
2	Tracheids	142 - 231
3	Fibres	257 - 312
4	Tracheary	260 – 313
5	Sclereids	172 – 249
6	Stone cells	35 - 71
7	Epidermal cells	56 - 60
8	Parenchyma cells	88 - 145
9	Unicellular trichomes	140 - 241
10	Uniseriate trichomes	77 - 150
11	Glandular trichomes	167 - 189
12	Stellate trichome	96 - 135
13	Prismatic calcium crystals	18- 27
14	Acicular crystals	80 - 94
15	Starch grains	18 – 31

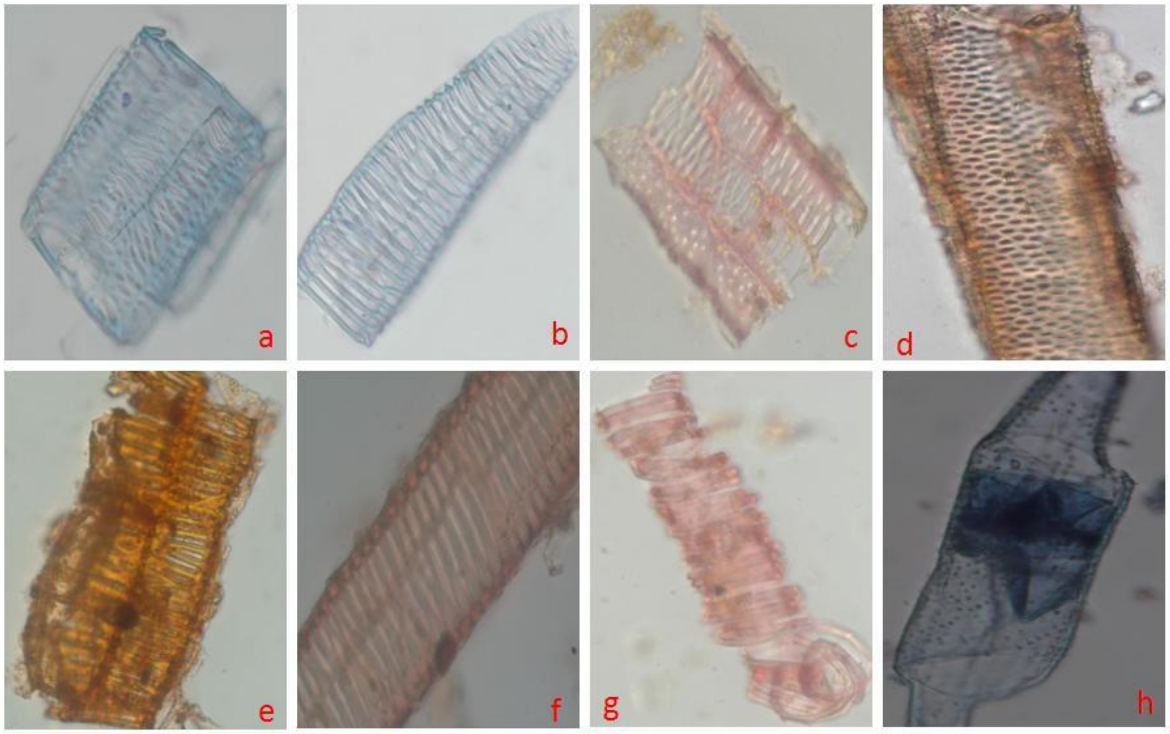


Fig. 1. Powder microscopic studies of Nilavembu kudineer

a – h. Different types of xylem thickenings. a. Reticulate thickening, b. Annular Thickening, c. Pitted, reticulate and annular thickening, d. Pitted thickening, e & f. Annular thickening, g. Spiral thickening, h. Tailed vessel

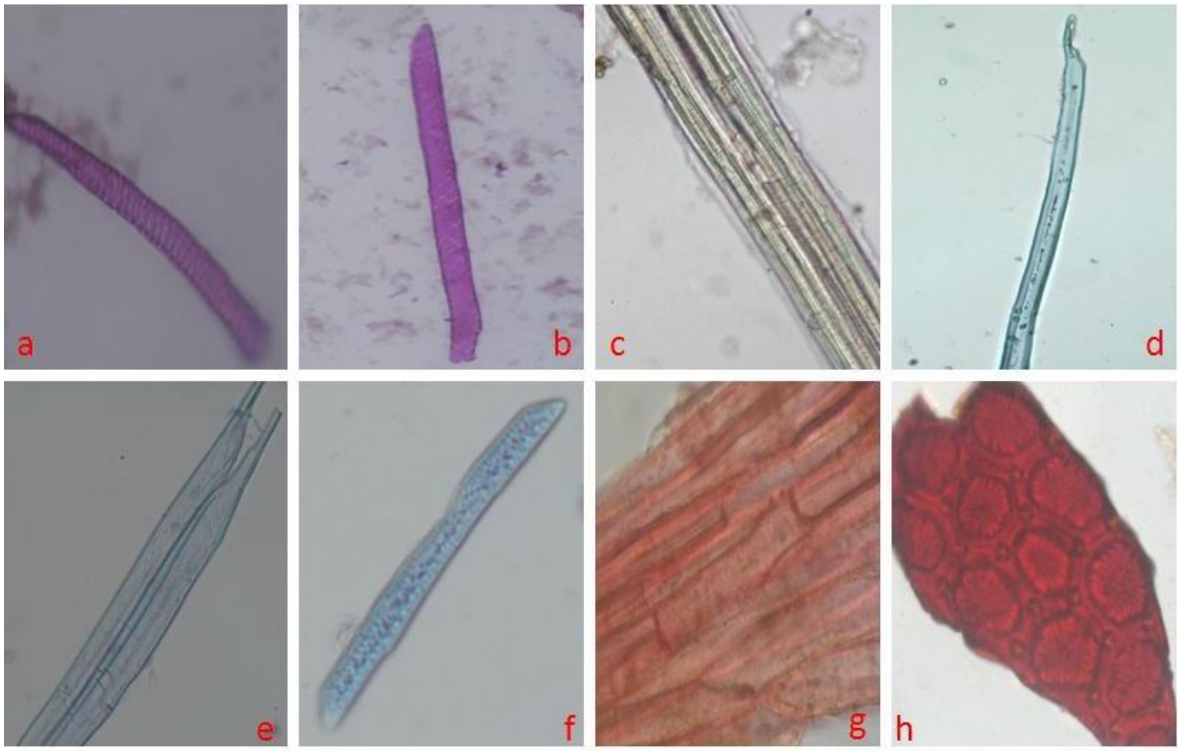


Fig. 2. Powder microscopic studies of Nilavembu kudineer

a.& b. Tracheids, c. Bundle of fibers, d. Libriform fiber, e. Tracheary fiber, f. Elongated sclereids, g. Sclerenchyma cells, h. Group of stone cells

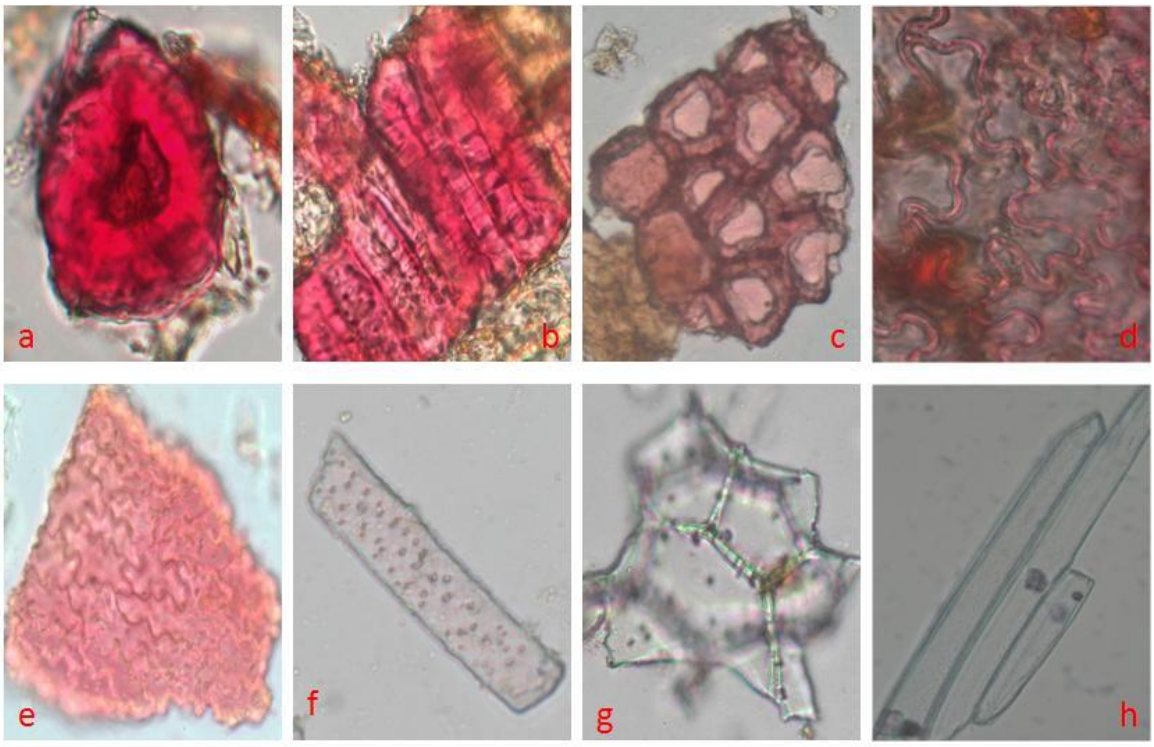


Fig. 3. Powder microscopic studies of Nilavembu kudineer
a.& b. Stone cells, c. Bundle of sclereids, d & e. Lignified wavy, epidermal cells, f. Parenchyma cells with pits, g Beaded cell wall, h. Elongated parenchyma cells

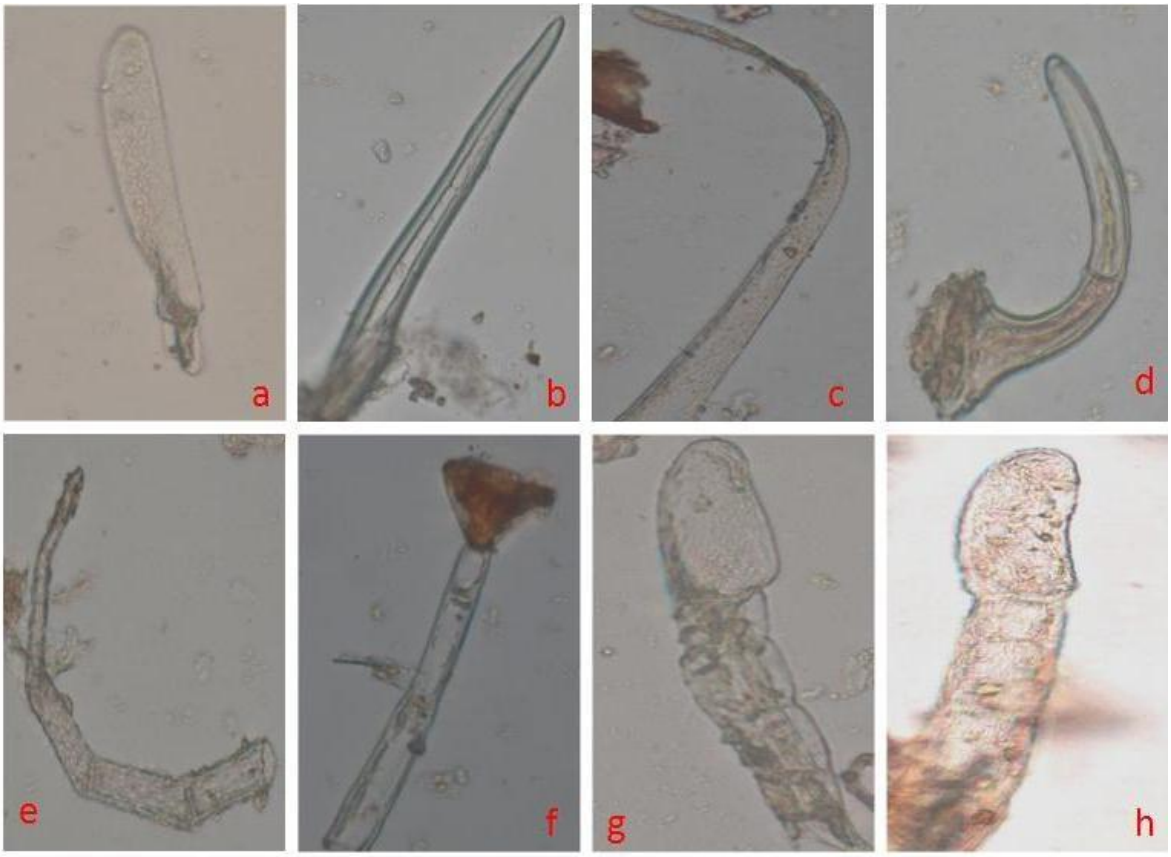


Fig. 4. Powder microscopic studies of Nilavembu kudineer

a - c. Unicellular trichome, warty surface (a), smooth surface (b) elongated (c), d & e. Uniseriate trichomes, smooth surface (d), warty surface (e), f. Uniseriate stalked unicellular headed glandular trichome, g & h. Unicellular stalked glandular trichome

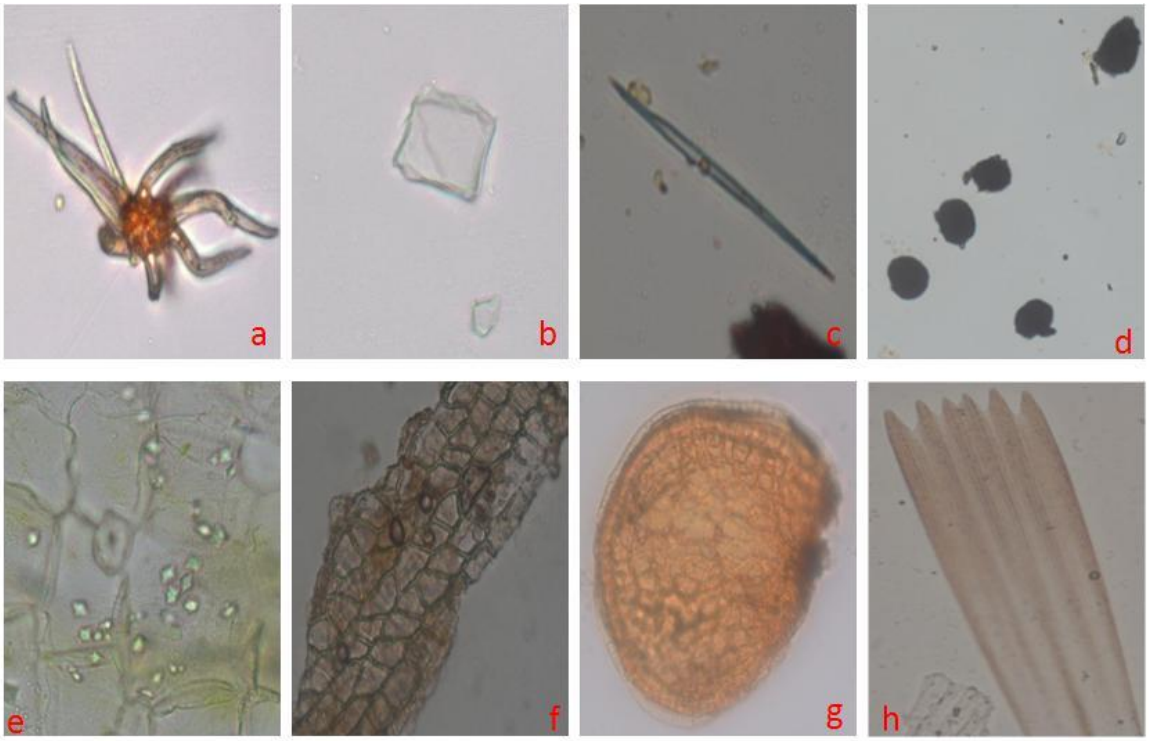


Fig. 5. Powder microscopic studies of Nilavembu kudineer
a. Stellate trichome, b. Prismatic calcium oxalate crystal, c. Acicular calcium oxalate crystal, d. Simple starch grains, e. Epidermal cell with calcium crystals, f. Thick walled parenchyma cells, g. Seed coat, h. Calyx.

Powder microscopy of ***Nilavembu Kudineer*** revealed the presence of all the ingredients mentioned in the Siddha text. The unique microscopic features of the ingredients of ***Nilavembu Kudineer*** observed were compared with the microscopic characters of individual ingredients which are mentioned in the Siddha Pharmacopoeia and other literature.

This literature comparison suggested presence of all the ingredients in ***Nilavembu Kudineer.*** Unique botanical characters observed in the present study for ingredients of ***Nilavembu Kudineer*** is discussed in sequel.

Tailed xylem vessels with pitted thickening, rectangular parenchyma cells, prismatic calcium oxalate crystals and oil globules (*Santalum album*); Tracheids fibres with branching and split ends, libriform, thin walled, tapering end with wide lumen, round to oval starch grains, prismatic calcium oxalate crystals, vessels with simple pitted and scalariform thickening, parenchyma cells with reticulation (*vilamichaiver - Plectranthes vettiveroides*).

Epidermal cells are wavy, sclerenchyma cells with narrow lumen, tracheids are thick walled, brown colour seeds, tricolpate pollen grains and group of fibres (*papatakam- Molluco cerviana*). Brown content, slightly elongatedand beaker shaped stone cells, stone cells, minute compound and simple starch grains (*milagu – Piper nigrum*). Round to oval shaped starch grains and groups of fibres (*vettiver- Vettiveria zizanioides*). Septate and crisscross fibres, vessels with reticulate and spiral thickening, brown content and striated starch grains (*chukku-Zingiber officinale*). Vessels with reticulate and simple pitted thickening, fibre like sclereid cells, narrow xylem vessels with scalariform thickening (k*oraikizhanku – Cyperus rotundus*). Acicular calcium oxalate crystals, xylem vessels with spiral and pitted thickening, unicellular and short and long uniseriate trichomes with bi and tri cellular, short and long uniseriate multicellular headed glandular trichome, spherical shaped pollen grains (*Nilavembu-Andrographis paniculata*). Polygonal cork cells, striated sclereids are isodiametric or in various shapes with round branched lumen, grouped fibres, xylem vessels with reticulate and bordered pitted thickenings (*peipudal – Trichosanthes cucumerina*).

Histochemical analysis using various chemical reagents revealed the presence of lignin, starch, oils, fats and resins. Presents of calcium oxalate crystal were also confirmed through this technique.

6.2Chemical Standardization Studies

The data of the results obtained on chemical standardization studies such as test for identity, purity and strength determined were also given Table11. Besides HPTLC and GC-MS analysis were also performed and the results were also given. XRF analysis was carried out to detect the presence of various metals and minerals. Microbial load and heavy metal analysis were also performed to check the contamination.

Table:11 Tests for identity, purity and strength

S. No	Parameters	Results	Range
1	Appearance	Brown coloured fine powder	-
2	pH (1% w/v solution)	6.82	4-14
3	Total Ash	30.13%w/w	1-25%
4	Acid Insoluble Ash	1.791%w/w	0.1-10%
5	Loss on Drying at 105°C	5.739%w/w	1-20%
6	Water Soluble Extractive(WSE)	81.07%w/w	4-85%
7	Alcohol Soluble Extractive(ASE)	3.589%w/w	4-85%

6.4Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of various bioactive compounds like **alkaloids, sterols, terpenoids, saponins, carbohydrates** and mucilage in ***Nilavembu Kudineer***. The tests for flavonoids, tannins and phenols gave negative results, which indicated their absence in ***Nilavembu Kudineer***.

Table:12 Estimation of Alkaloids, Carbohydrates and Mucilage

S No	Constituents estimated	Results
1	Alkaloids	0.9322%
2	Carbohydrates	35.28%
3	Mucilage	0.1692%

6.5 HPTLC analysis

The HPTLC analysis of ***Nilavembu Kudineer*** revealed the presence of 11 spots with R_f at 0.31, 0.55, 0.58, 0.65, 0.70, 0.77, 0.82, 0.88, 0.92, 0.95 and 0.97 under 254 and 366 nm. The R_f value 0.70 indicates the presence of Andrographolide, the marker compound.

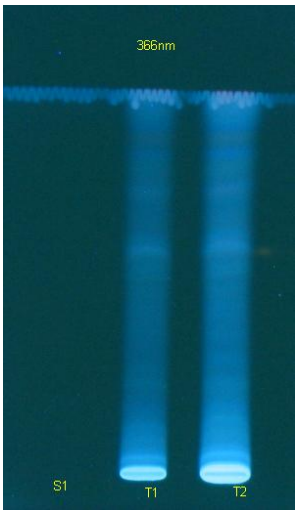
HPTLC Finger printing studies

HPTLC FINGERPRINTING PROFILE OF *Nilavembu Kudineer* Churnam

PHOTO DOCUMENTATION UNDER UV

AT 254nm

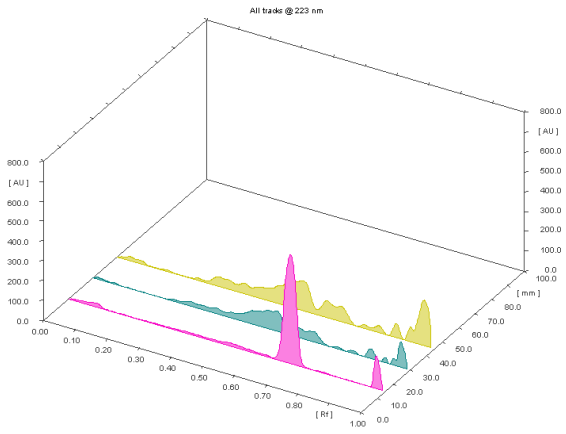
AT 366nm



TLC DETAILS

S1-10µl of standard Andrographolide, T1-10µl of sample Solution, T2-10µl of sample Solution

3D DISPLAY AT 254nm



PEAK DISPLAY (10µl of Standard)

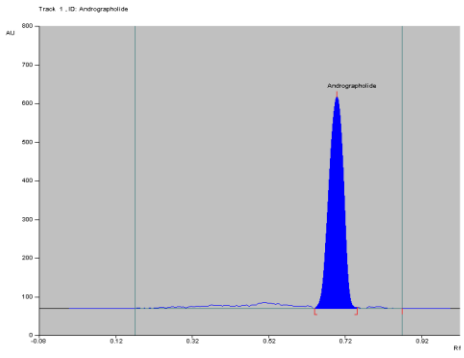


FIG-I

PEAK DISPLAY (10µl of Sample)

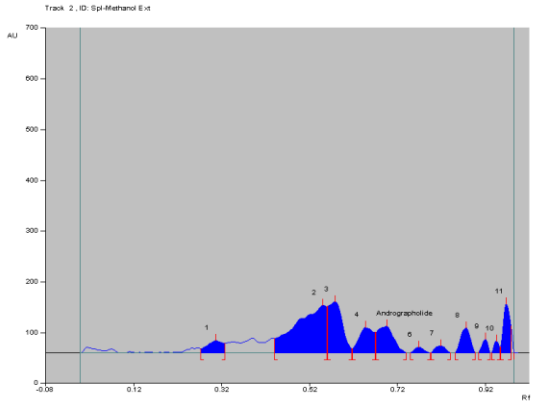
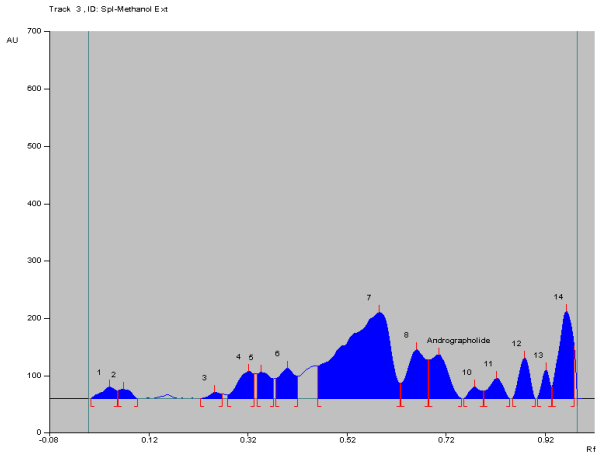


FIG-II

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.64	0.3	0.70	547.7	100.00	0.75	2.6	15359.8	100.00	Andrographolide

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.28	8.4	0.31	24.3	4.50	0.33	18.6	637.5	4.88	unknown *
2	0.44	28.5	0.55	93.6	17.31	0.56	91.3	4746.2	36.33	unknown *
3	0.56	91.7	0.58	100.9	18.67	0.62	8.7	2375.3	18.18	unknown *
4	0.62	8.8	0.65	49.8	9.21	0.67	40.2	1214.1	9.29	unknown *
5	0.67	40.3	0.70	52.6	9.74	0.74	0.2	1422.4	10.89	Andrographolide
6	0.75	0.3	0.77	11.3	2.08	0.80	0.0	171.5	1.31	unknown *
7	0.80	0.6	0.82	13.7	2.53	0.84	0.2	227.5	1.74	unknown *
8	0.85	0.6	0.88	48.9	9.04	0.90	0.9	782.3	5.99	unknown *
9	0.91	0.8	0.92	26.5	4.90	0.93	0.2	251.7	1.93	unknown *
10	0.94	0.0	0.95	23.0	4.25	0.96	10.5	185.4	1.42	unknown *
11	0.96	12.0	0.97	96.1	17.77	0.98	52.3	1050.4	8.04	unknown *

PEAK DISPLAY (20µl of Sample)



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	0.5	0.04	20.0	2.40	0.06	14.0	426.4	1.73	unknown *
2	0.06	14.2	0.07	16.2	1.94	0.10	0.1	294.9	1.20	unknown *
3	0.23	0.0	0.26	11.0	1.32	0.27	7.8	175.6	0.71	unknown *
4	0.28	6.5	0.32	47.6	5.71	0.33	43.2	1084.4	4.40	unknown *
5	0.34	43.3	0.35	46.0	5.52	0.37	34.6	915.7	3.71	unknown *
6	0.38	35.5	0.40	52.5	6.30	0.42	38.7	1281.6	5.20	unknown *
7	0.46	56.6	0.59	150.1	18.01	0.63	26.5	10561.7	42.84	unknown *
8	0.63	26.8	0.66	85.1	10.21	0.69	67.2	2285.7	9.27	unknown *
9	0.69	67.2	0.71	76.6	9.19	0.75	0.2	2010.0	8.15	Andrographolide
10	0.76	0.2	0.78	20.4	2.44	0.80	12.9	331.8	1.35	unknown *
11	0.80	13.0	0.83	35.0	4.20	0.85	0.4	671.0	2.72	unknown *
12	0.86	0.5	0.88	70.8	8.50	0.90	0.1	1080.8	4.38	unknown *
13	0.91	1.3	0.92	49.9	5.99	0.94	19.8	554.8	2.25	unknown *
14	0.94	20.8	0.97	152.2	18.26	0.98	92.8	2980.3	12.09	unknown *

The HPTLC analysis of ***Nilavembu Kudineer*** revealed the presence of 11 spots with R_f at 0.31, 0.55, 0.58, 0.65, 0.70, 0.77, 0.82, 0.88, 0.92, 0.95 and 0.97 under 254 and 366 nm. The R_f value 0.70 indicates the presence of Andrographolide, the marker compound.

6.6 GCMS –Studies:

Chromatogram

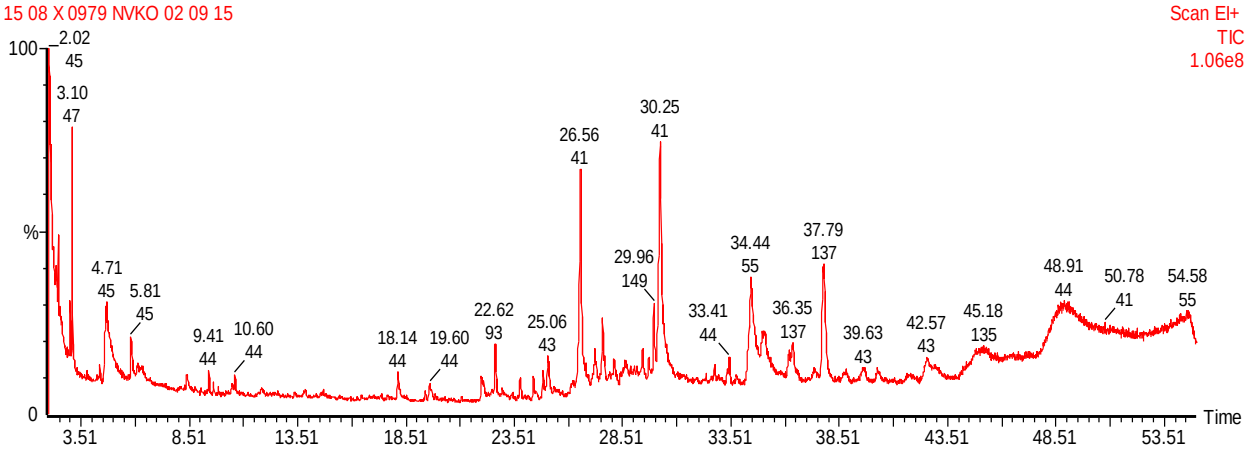


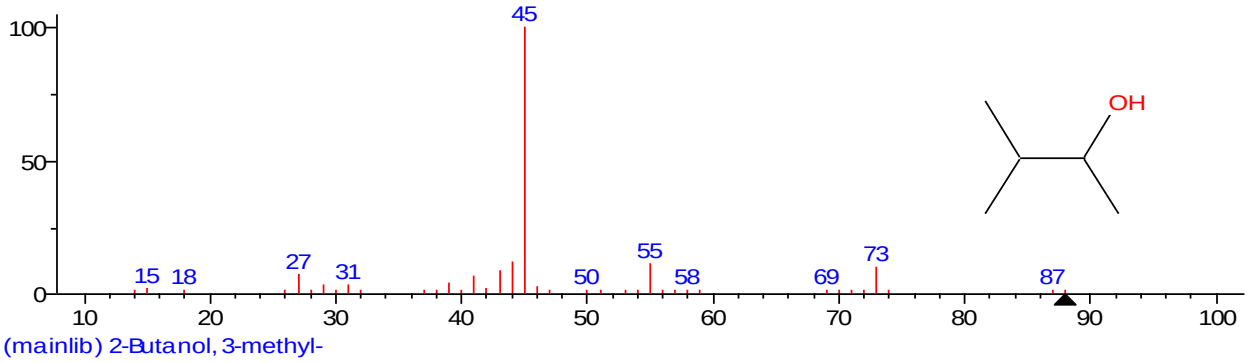
Table:13 List of compounds identified in GCMS

S.No.	Peak Name	Retention time	Peak area	%Peak area
1.	Name: 2-Butanol, 3-methyl- Formula: $C_5H_{12}O$ MW: 88	4.71	277476	0.4246
2.	Name: 1,3-Propanediamine, N-methyl- Formula: $C_4H_{12}N_2$ MW: 88	8.38	406999	0.6228
3.	Name: Homopiperazine Formula: $C_5H_{12}N_2$ MW: 100	10.49	238174	0.3645
4.	Name: Decanal Formula: $C_{10}H_{20}O$ MW: 156	10.60	306506	0.4691
5.	Name: Piperonal Formula: $C_8H_6O_3$ MW: 150	13.84	101365	0.1551
6.	Name: Phenol, 2,4-bis(1,1-dimethylethyl)- Formula: $C_{14}H_{22}O$ MW: 206	18.14	912183	1.3959
7.	Name: Bicylo[4.1.0]heptane, 7-bicyclo[4.1.0]hept-7-ylidene- Formula: $C_{14}H_{20}$ MW: 188	19.40	163449	0.2501
8.	Name: Tridecanoic acid Formula: $C_{13}H_{26}O_2$ MW: 214	19.60	689803	1.0556

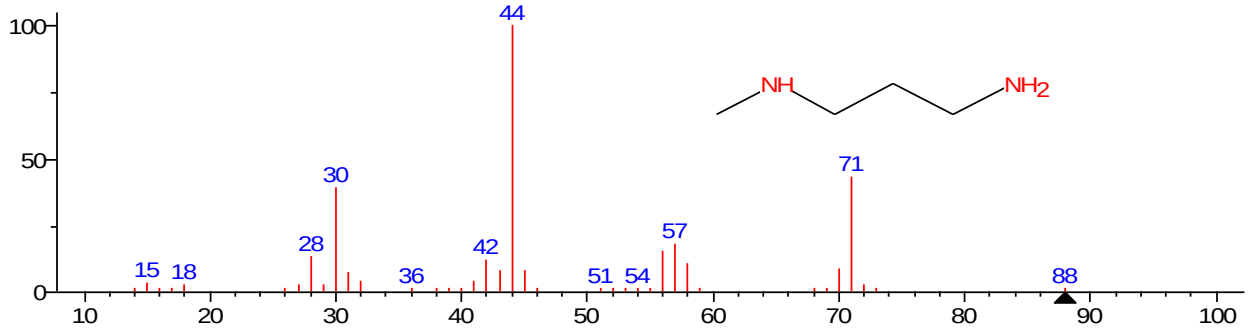
9.	Name: Tricyclo[2.2.1.0(2,6)]heptane-3-methanol, 2,3-dimethyl- Formula: $C_{10}H_{16}O$ MW: 152 Teresantalol	21.08	91218	0.1396
10.	Name: 2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)- Formula: $C_{11}H_{14}O_3$ MW: 194 Zingerone	21.97	238658	0.3652
11.	Name: Santalol, cis,à- Formula: $C_{15}H_{24}O$ MW: 220	22.10	31752	0.0486
12.	Name: Santalol Formula: $C_{15}H_{24}O$ MW: 220	22.62	1168835	1.7887
13.	Name: 2-Decanone Formula: $C_{10}H_{20}O$ MW: 156	22.94	132709	0.2031
14.	Name: à-Santalol Formula: $C_{15}H_{24}O$ MW: 220	23.77	522339	0.7993
15.	Name: 7-Acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo[4.3.0]nonane Formula: $C_{15}H_{26}O_2$ MW: 238	24.39	512413	0.7842
16.	Name: 9H-Pyrido[3,4-b]indol-7-ol, 1-methyl- Formula: $C_{12}H_{10}N_2O$	24.83	562926	0.8615

	MW: 198 Name: 4-Phenoxybenzaldehyde Formula: $C_{13}H_{10}O_2$ MW: 198			
17.	Name: Tetradecanoic acid Formula: $C_{14}H_{28}O_2$ MW: 228	25.06	1492063	2.2833
18.	Name: 1H-3a,6-Methanoazulene-3-carboxylic acid, octahydro-7,7-dimethyl-8-methylene-, [3S-(3à,3aà,6à,8aà)]- Formula: $C_{15}H_{22}O_2$ MW: 234 Vetivenic acid	26.56	8733962	13.3658
19.	Name: 3,5,7-Nonatrien-2-one, 8-methyl-7-(1-methylethyl)-, (E,E)- Formula: $C_{13}H_{20}O$ MW: 192	28.10	798144	1.2214
20.	Name: Andrographolide Formula: $C_{20}H_{30}O_5$ MW: 350	28.60	1384370	2.1185
21.	Name: Naphthalene, decahydro-1,1-dimethyl- Formula: $C_{12}H_{22}$ MW: 166	29.44	897201	1.3730
22.	Name: Bicyclo[2.2.1]heptane, 2-methyl-3-methylene-2-(4-methyl-3-pentenyl)-, (1S-endo)- Formula: $C_{15}H_{24}$ MW: 204(Epi-á-Santalene)	29.71	354926	0.5432

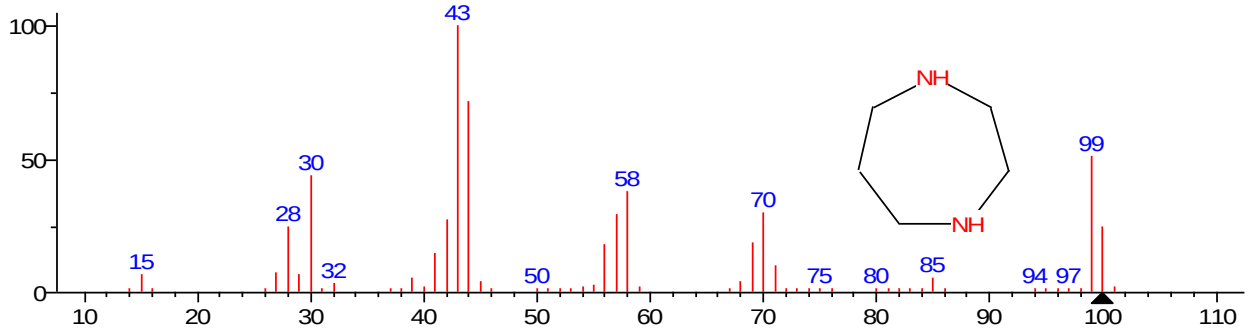
23.	Name: n-Hexadecanoic acid Formula: $C_{16}H_{32}O_2$ MW: 256	30.25	9997901	15.3000
24.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	33.41	409889	0.6273
25.	Name: 2-Methyl-Z,Z-3,13-octadecadienol Formula: $C_{19}H_{36}O$ MW: 280	34.44	8832656	13.5168
26.	Name: (-)-Nortrachelogenin Formula: $C_{20}H_{22}O_7$ MW: 374	36.19	755638	1.1564
27.	Name: Gingerol Formula: $C_{17}H_{26}O_4$ MW: 294	36.35	1552711	2.3762
28.	Name: Gingerol Formula: $C_{17}H_{26}O_4$ MW: 294	37.79	5788098	8.8577
29.	Name: Acetic acid, 1-[2-(2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-ethyl]-vinyl ester Formula: $C_{16}H_{26}O_2$MW: 250	42.57	1924017	2.9444
30.	Name: Piperine Formula: $C_{17}H_{19}NO_3$MW: 285	48.89	16067177	24.5880



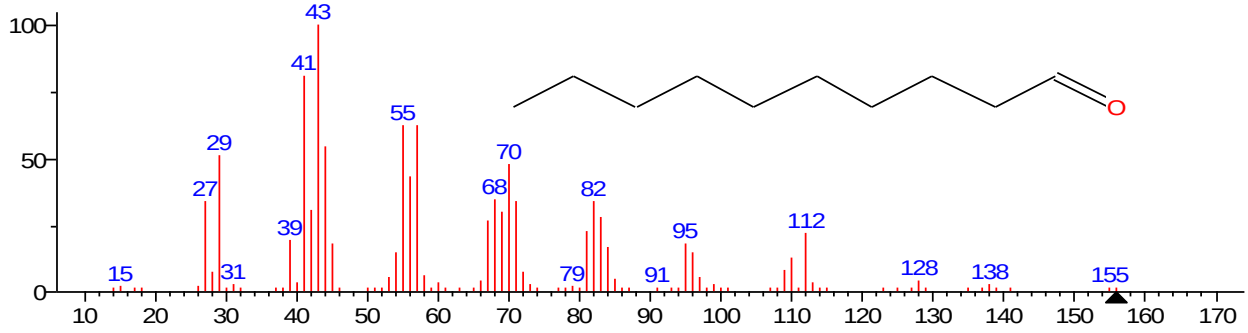
(mainlib) 2-Butanol, 3-methyl-



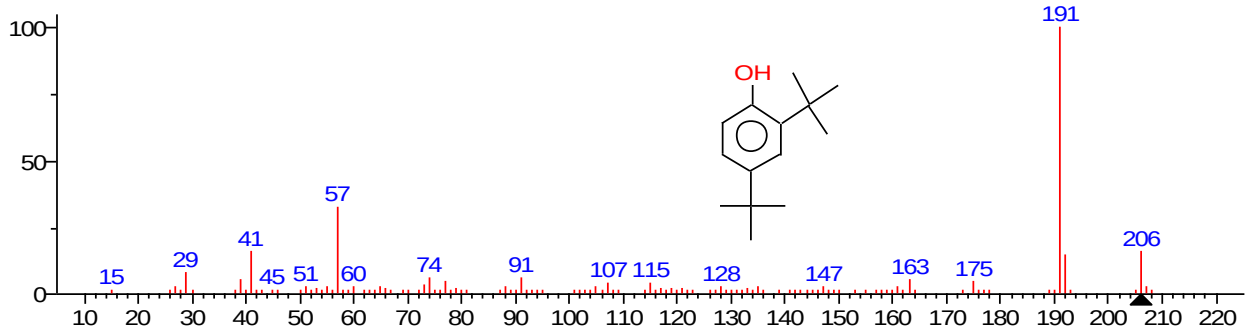
(mainlib) 1,3-Propanediamine, N-methyl-



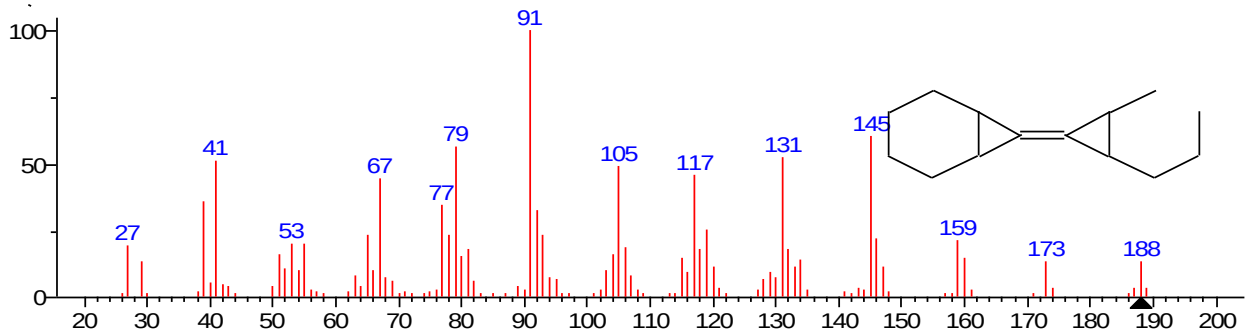
(mainlib) Homopiperazine



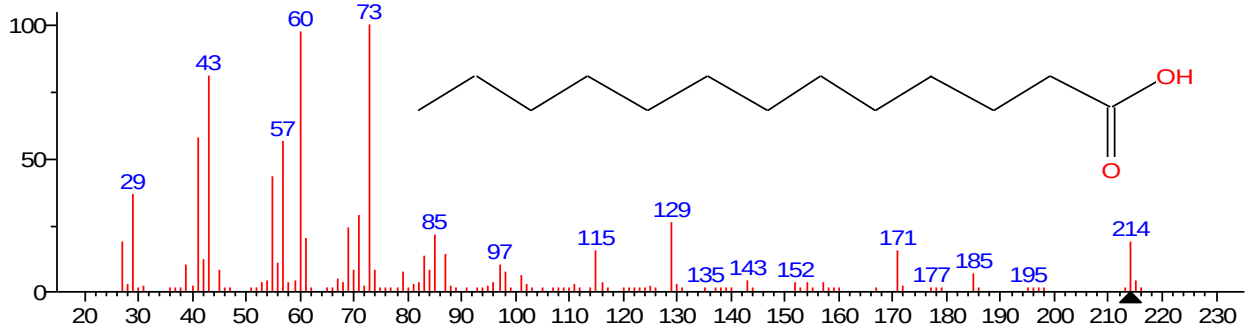
(mainlib) Decanal



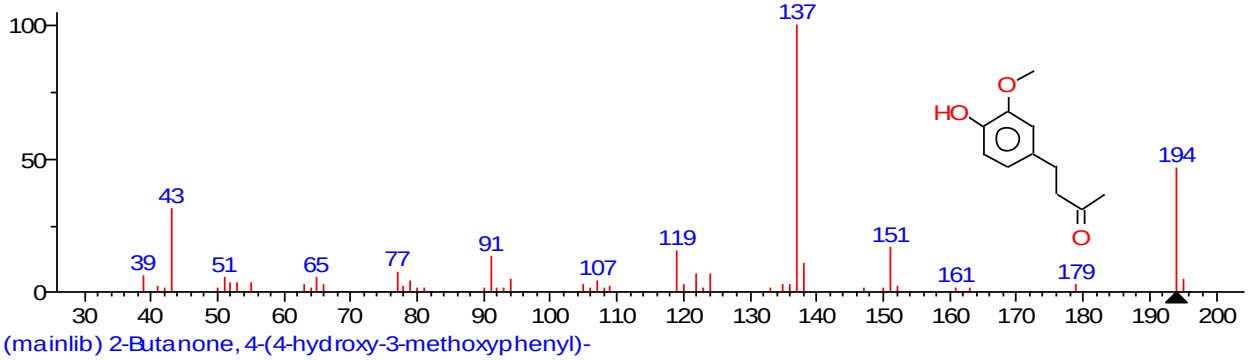
(mainlib) Phenol, 2,4-bis(1,1-dimethylethyl)-



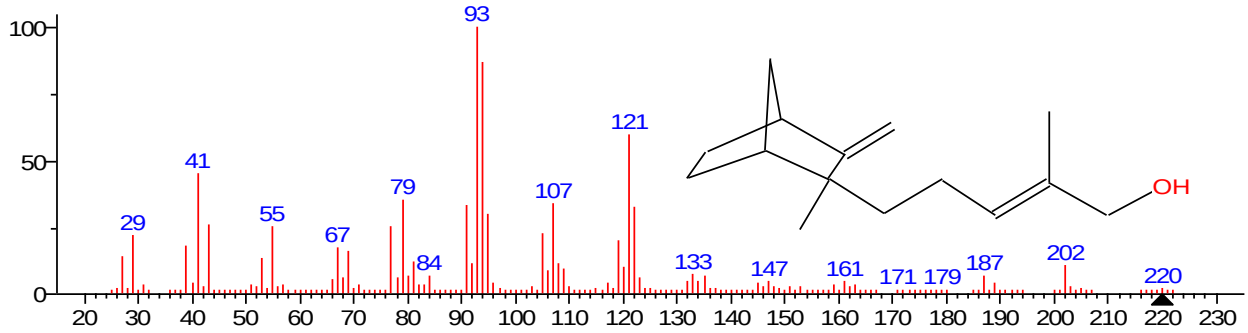
(mainlib) Bicylo[4.1.0]heptane, 7-bicyclo[4.1.0]hept-7-ylidene-



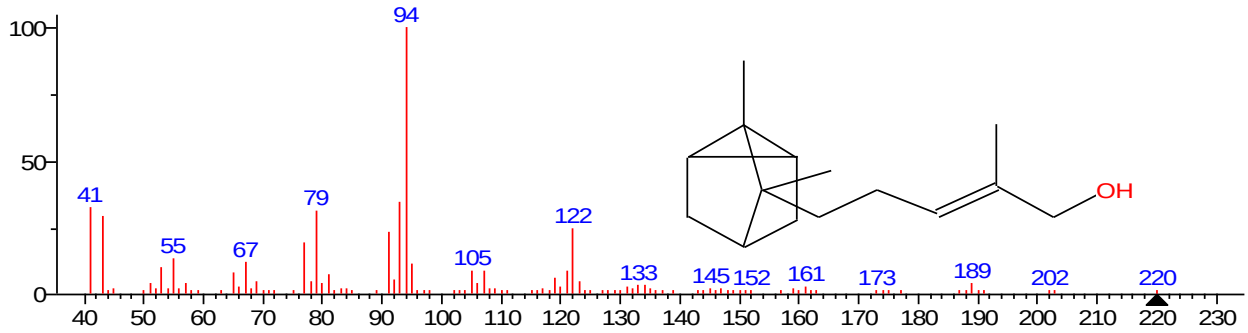
(mainlib) Tridecanoic acid



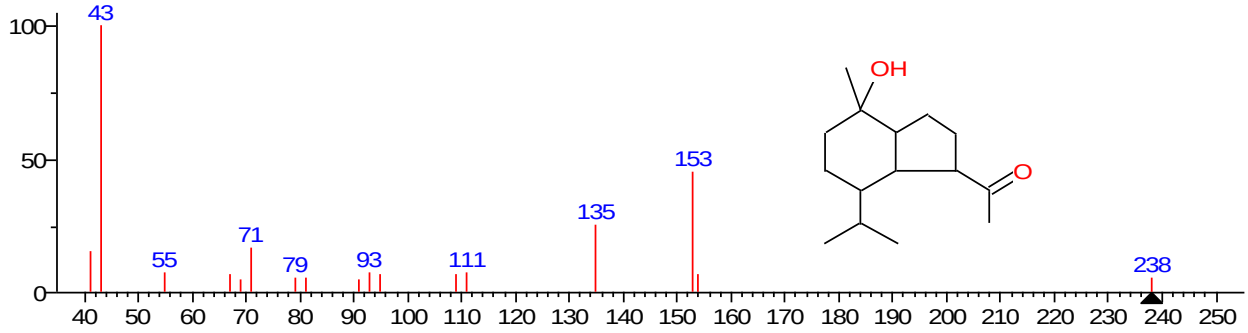
(mainlib) 2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)-



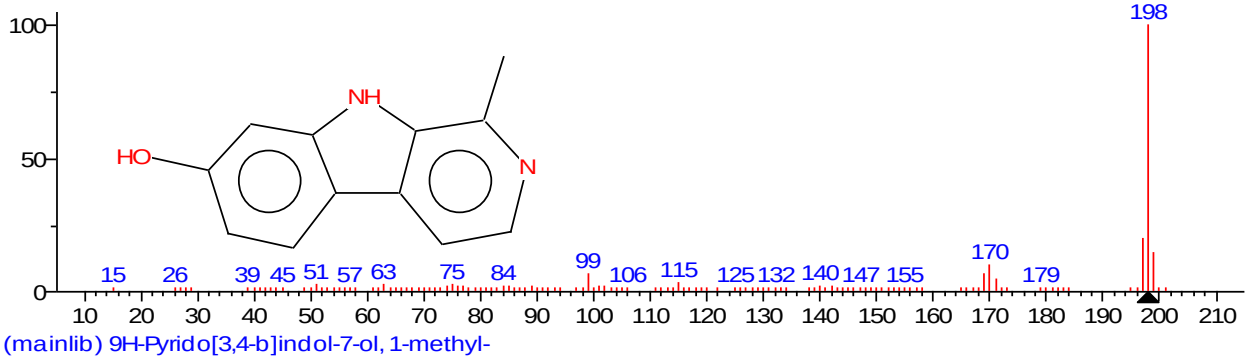
(mainlib) Santalol



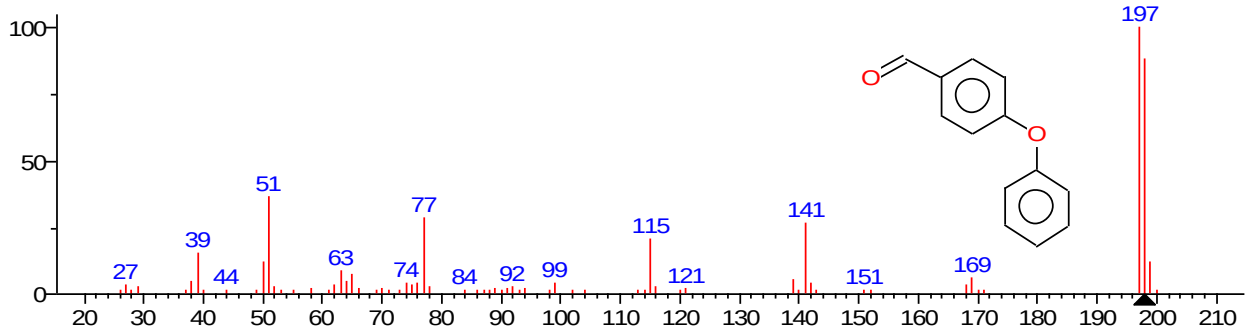
(mainlib) à-Santalol



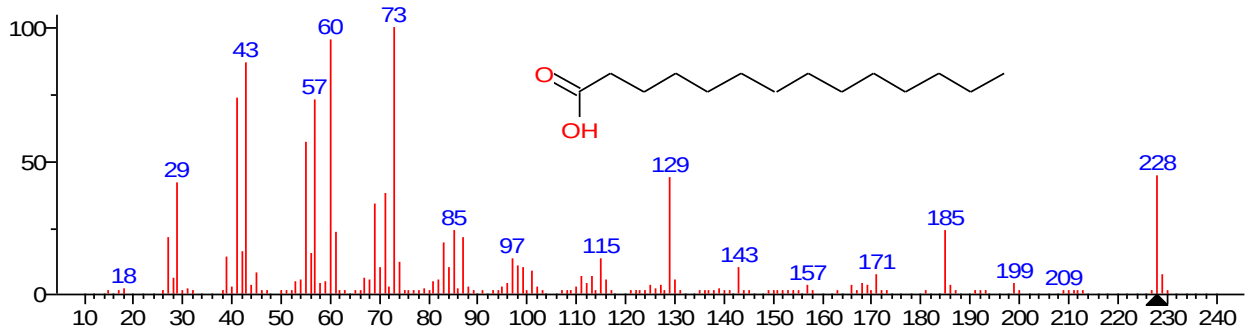
(mainlib) 7-Acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo[4.3.0]nonane



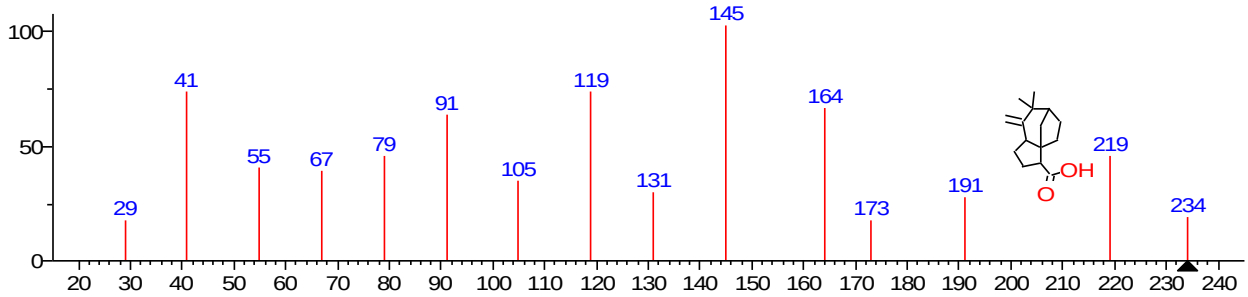
(mainlib) 9H-Pyrido[3,4-b]indol-7-ol, 1-methyl-



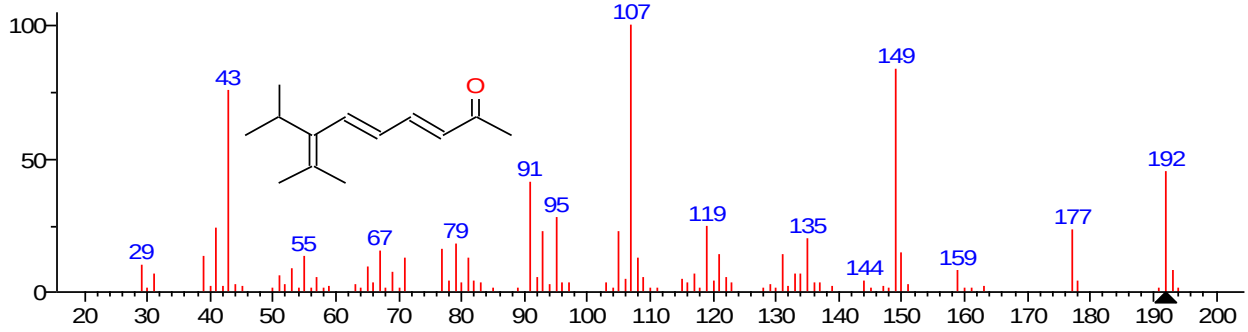
(mainlib) 4-Phenoxybenzaldehyde



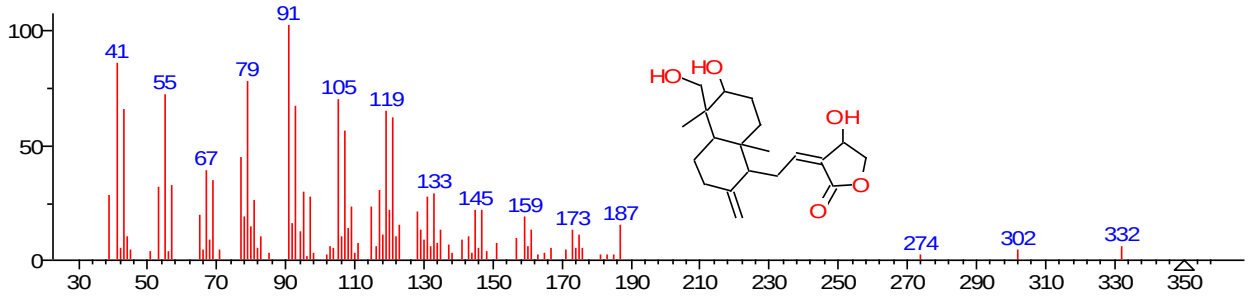
(mainlib) Tetradecanoic acid



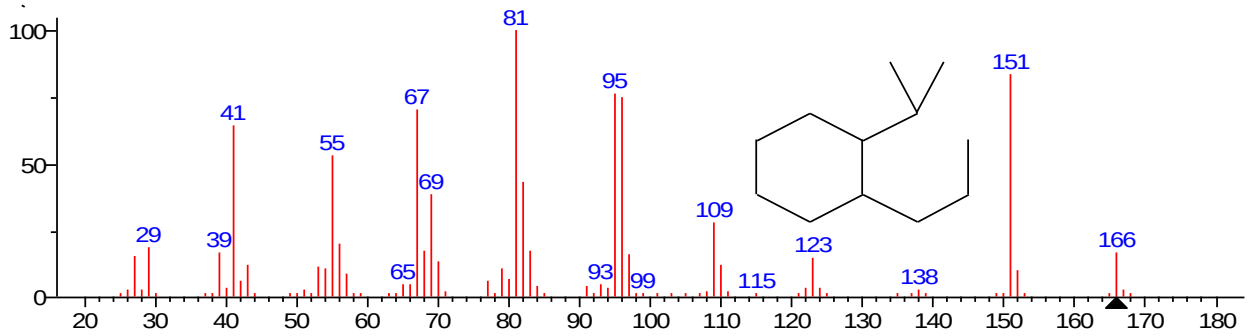
(mainlib) 1H-3a,6-Methanoazulene-3-carboxylic acid, octahydro-7,7-dimethyl-8-methylene-, [3S(3à,3aà,6à,8aà)]-



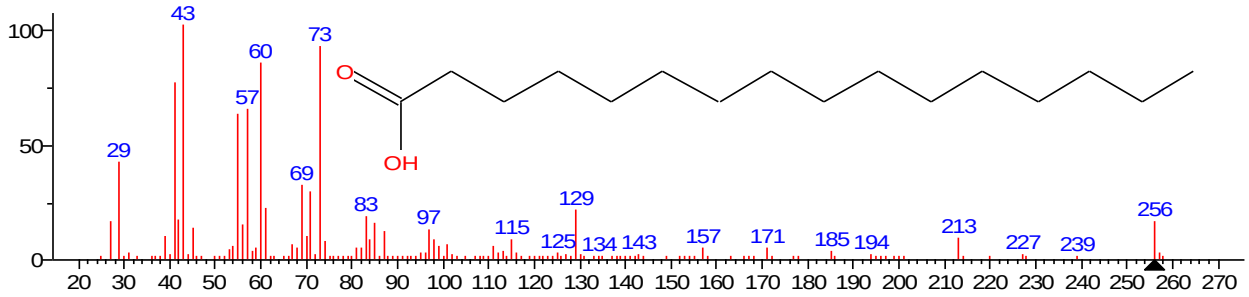
(mainlib) 3,5,7-Nonatrien-2-one, 8-methyl-7-(1-methylethyl)-, (E,E)-



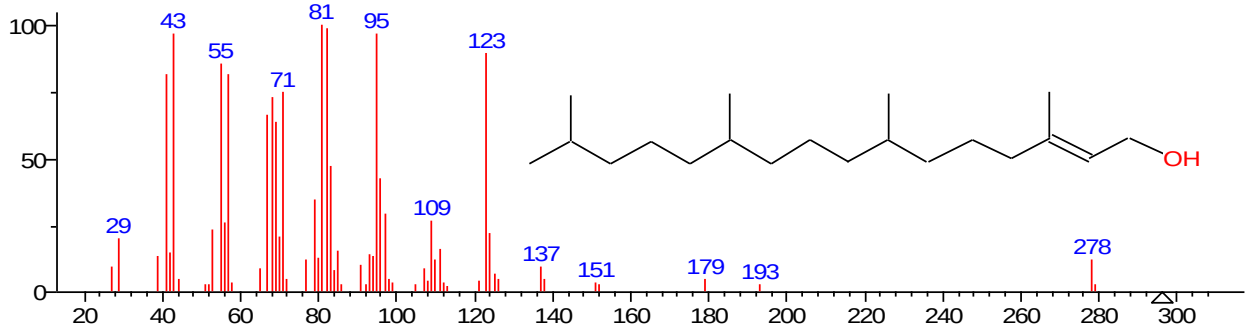
(mainlib) Andrographolide



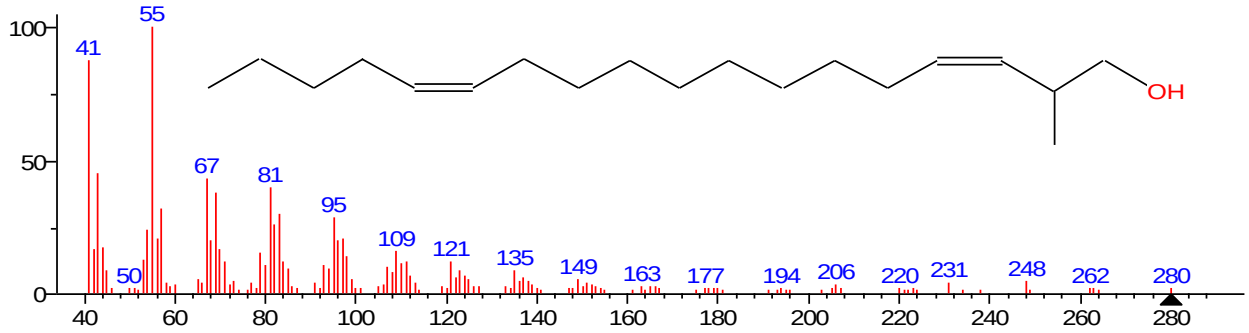
(mainlib) Naphthalene, decahydro-1,1-dimethyl-



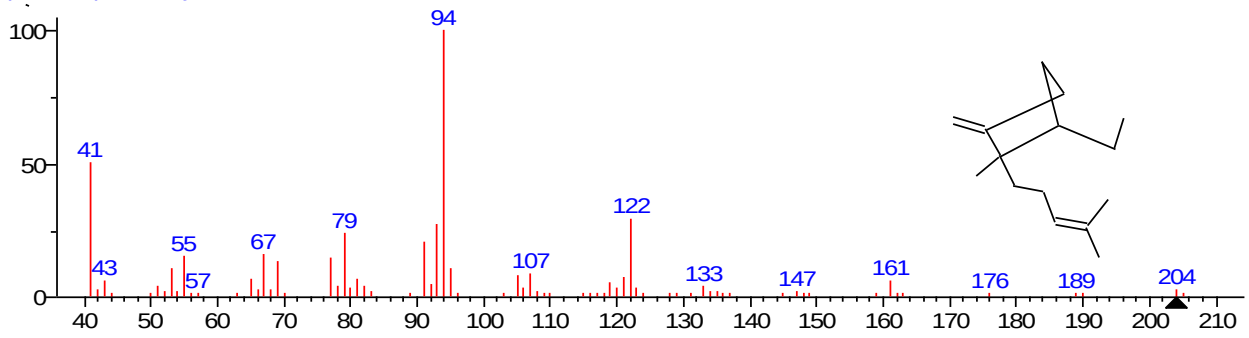
(mainlib) n-Hexadecanoic acid



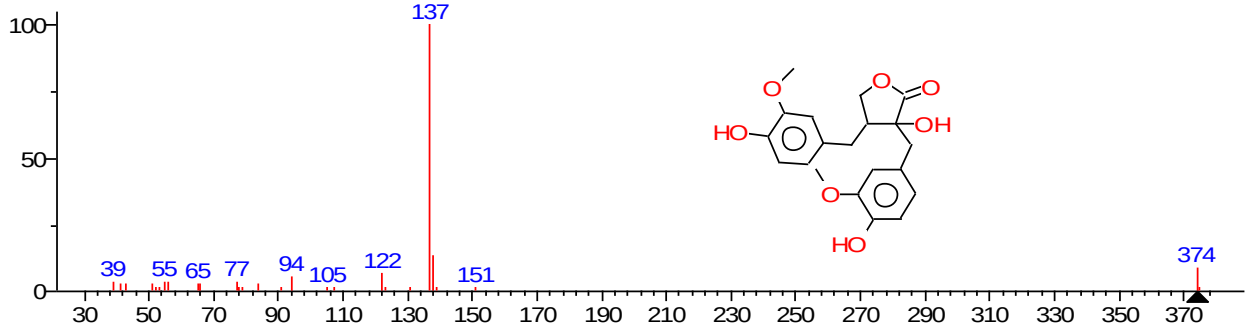
(mainlib) 3,7,11,15-Tetramethyl-2-hexadecen-1-ol



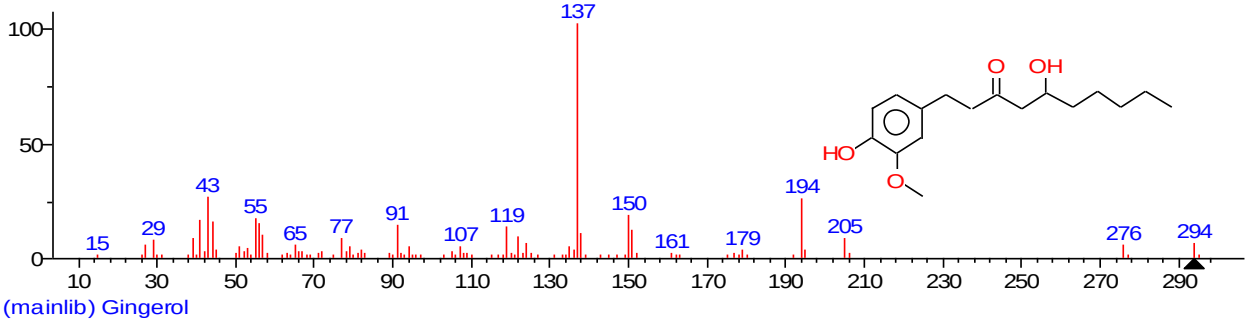
(mainlib) 2-Methyl-Z,Z-3,13-octadecadienol



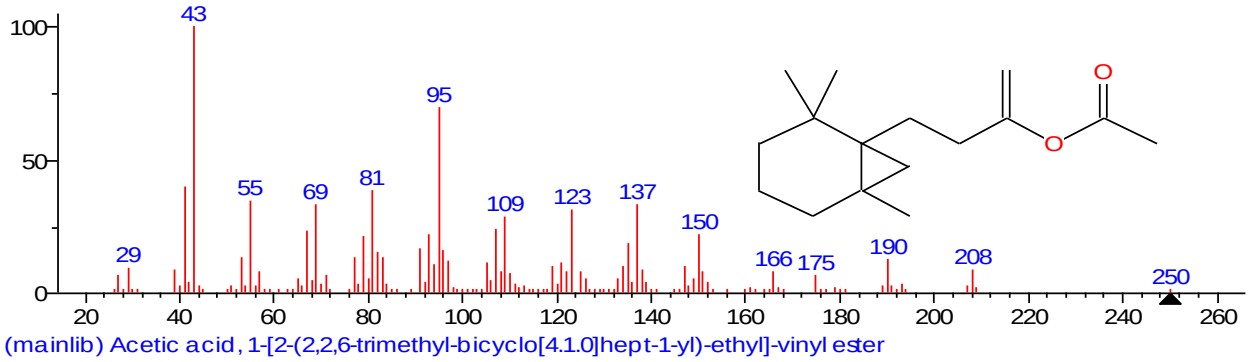
(mainlib) Bicyclo[2.2.1]heptane, 2-methyl-3-methylene-2-(4-methyl-3-pentenyl)-, (1S-endo)-

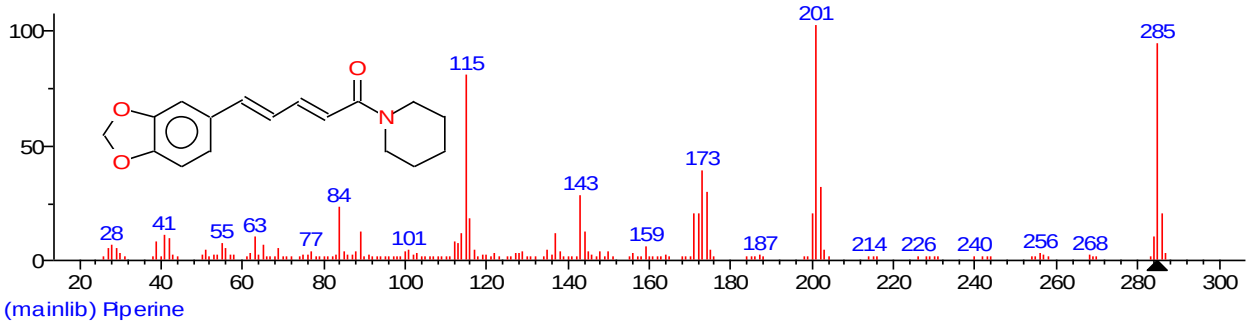


(mainlib) (-)-Nortrachelogenin



(mainlib) Gingerol

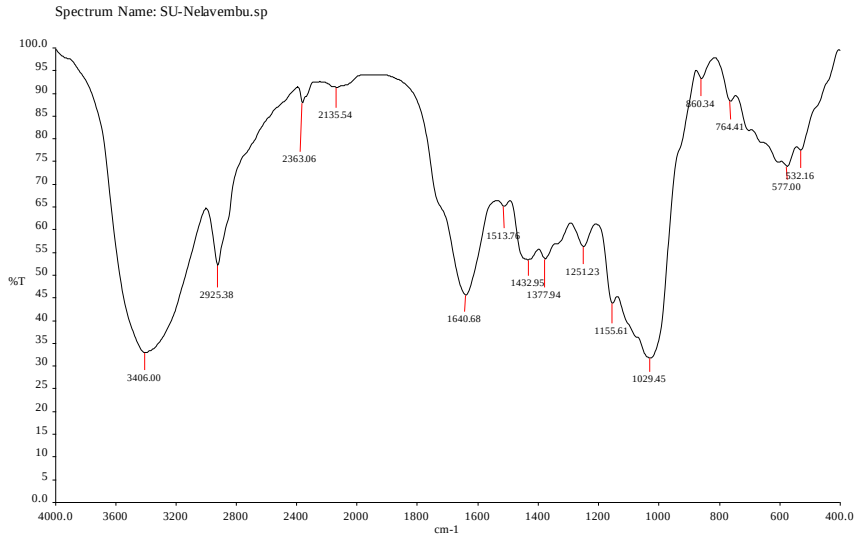




The data obtained on GCMS analysis of ***Nilavembu Kudineer*** showed fragmentation pattern of flavonoids and piperine which is evident in the chromatogram also.

The GC-MS analysis further showed the presence of vetiveric acid, palmitic acid, gingerol, santalol, piperine and andrographolide in the selected formulation suggesting the presence of few ingredients such as Andrographis, Vettiver, Ginger, Santalum album and Pepper. Some of the anti-diabetic molecules such as vettiveric acid, piperine and andrographolide were also identified in the GC-MS analysis.

6.7 FTIR spectrum of the *Nilavembu Kudineer*



Characteristic peaks were observed at 3406^{cm-1} indicates presents of alcohols and amines as these compounds show a conspicuous OH or NH stretching absorption at 3000-3700^{cm-1} (to the left of the hydrocarbon CH stretch). This suggests presence of amines in the sample.

A peak at 1155.61^{cm-1} is suggestive of presence of C-O stretch. A peak at 1640.68^{cm-1} indicates carbonyl (C=O) stretch. A peak at 2925.38^{cm-1} indicates CH stretch peak at 1377.94^{cm-1} indicates Methyl C-H asym./sym. Bend of the compounds present in the sample. Peak at 1029.45^{cm-1} indicates presence of C-N bond. Peak at 764.41^{cm-1} indicates the presence of mono-substituted benzene ring.

Sharp peaks at various position as given below indicate presence of bonds as specified which could be used as finger printing for the preparation:

Table:14 FTIR Analysis

Position	Bond
764.41^{cm-1}	Disubstituted benzene ring
1029.45^{cm-1}	Primary amine,CN stretch
1155.61^{cm-1}	Amine based
1377.94^{cm-1}	Methyl C-H asym./sym
1640.68^{cm-1}	C=C stretch
2925.38^{cm-1}	Methylene CH stretch
3406^{cm-1}	Hydroxyl group,OH stretch

6.8 HEAVY METAL ANALYSIS

Table: 15 Heavy Metal Analysis

Element	Units in ppm	Permissible level(ppm)
Pb	BDL	10
Hg	BDL	1
As	BDL	10
Cd	BDL	0.3

Heavy metal analysis revealed that heavy metals like Pb, Hg, As and Cd were present in permissible limits

6,9 Microbial Analysis

Table:16 Total Microbial Count for *Nilavembu Kudineer*

S.No	Bacterial Name	W.H.O. Limit	Cells in Sample/g	Interference
1.	*E.coli*	10^2	10 X 10^2	Within Limit
2.	*Salmonella sp.*	Absence	-	Within Limit
3.	*Shigella sp.*	Absence	-	Within Limit
4.	*Enterobacteria sp.*	10^4	-	Within Limit
5.	Total Heterotrophic Bacteria	10^7	112 X 10^4	Within Limit
6.	Yeast and Mould	10^4	19 x 10^1	Within Limit

Microbial analysis suggested that the drug is free from microbial contamination

6.10 XRF Analysis

Elemental form of various metals and minerals detected were given in Table 17

Table: 17 XRF Analysis

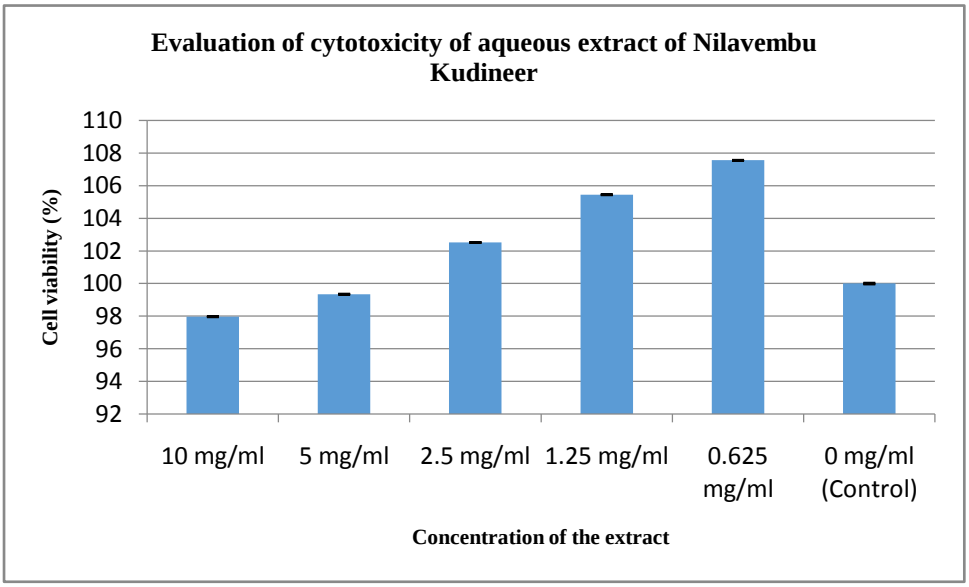
Formula	Concentration (%)
K	48.34
Ca	18.11
Cl	17.76
Mg	3.94
Si	3.54
S	2.07
P	1.74
Fe	1.47
Al	0.96
Na	0.68
Pb	0.62
Ti	0.18
Mn	0.16
Cu	0.14
Pd	0.11
Zn	0.09
Mo	0.05
Sr	0.03
Zr	48 PPM

6.11 In vitro studies

***In vitro* cytotoxicity studies:**

Result

Based on the results obtained from the present study, the aqueous extract of ***Nilavembu Kudineer*** was not toxic at a concentration range of 0.625 – 10 mg/ml in cell line model.



Cytotoxicity of aqueous extract of

Nilavembu

Table:18Cell viability(MMTassay)

	Extract conc (mg/ml)					Control
Rep	10	5	2.5	1.25	0.625	0
A	0.147	0.151	0.165	0.167	0.163	0.171
B	0.156	0.146	0.153	0.168	0.172	0.169
C	0.141	0.142	0.164	0.163	0.169	0.161
D	0.157	0.160	0.154	0.156	0.166	0.189
E	0.165	0.159	0.152	0.173	0.171	0.103
F	0.157	0.165	0.156	0.154	0.162	0.164
G	0.133	0.142	0.157	0.149	0.151	0.108
H	0.149	0.157	0.160	0.167	0.169	0.165
Avg	0.151	0.153	0.158	0.162	0.165	0.154
SD	0.010	0.009	0.005	0.008	0.007	0.031
Cell viability (%)	97.967	99.350	102.520	105.447	107.561	100.000

Based on the data obtained in the in vitro cytotoxicity studied it is concluded that the aqueous extract of ***Nilavembu Kudineer*** is not toxic at a concentration range of 0.625 – 10 mg/ml in cell line model.

6.12 PRE CLINICAL STUDIES

Acute oral toxicity study

Mortality

No mortality was recorded **(Table 19)**.

Body Weight

The animals treated with ***Nilavembu Kudineer*** did not show any significant change in body weight gain on day 7 and 14 when compared with Day 0. **(Table 20)**.

Feed Intake

The daily feed intake of rats remained unaffected throughout the experimental period **(Table 21).**

Toxicity Signs

No visible signs of toxicity such as changes in respiration, circulation, autonomic and central nervous system, behavioral pattern were observed during the entire study period. **(Table 22)**.

Gross Pathology

No test compound related findings were observed at necropsy. All gross observations were agonal in nature and bore no relation to treatment with the test substance **(Table 23)**. All animals survived until the end of study.

Results

Table: 19 Mortality Data

Details of the Group	**Total no. of rats treated**	**Dose (mg/kgb.w.)**	**Percent mortality (upto 14 days)**
Nilavembu Kudineer treated	5 Female	2000	0

b.w. - Body Weight.

Table: 20 Weekly Mean Body Weight Changes In Rats

Animal ID	Sex	Body Weight (g)		
		Day 0	Day 7	Day 14
6022	Female	183.22	186.99	190.01
6023	Female	171.07	175.23	177.93
6024	Female	174.02	184.47	188.29
6025	Female	169.82	173.08	174.20
6026	Female	178.06	182.64	188.10
Mean		175.24	180.48	183.71
Standard deviation		5.47	6.03	7.14

Table: 21 Daily Feed Intake

Animal	Sex	Days													
		0	1	2	3	4	5	6	7	8	9	10	11	12	13
6022	**F**	16.76	17.09	18.28	15.23	19.13	16.83	16.02	15.98	16.43	13.97	17.97	15.14	16.00	15.83
6023	**F**	16.95	16.32	21.03	16.98	17.54	14.74	18.39	15.88	18.41	15.70	17.26	16.47	17.94	17.63
6024	**F**	17.57	14.91	19.33	15.30	19.29	15.53	14.55	12.15	18.27	16.55	16.47	15.29	17.28	17.32
6025	**F**	16.37	15.96	18.15	15.82	17.04	15.05	18.89	16.19	18.30	16.29	16.76	16.50	16.30	16.97
6026	**F**	17.46	14.32	20.33	15.72	19.75	17.24	18.63	14.96	18.88	17.36	15.46	17.41	16.02	18.36
Mean		17.02	15.72	19.42	15.81	18.55	15.88	17.30	15.03	18.06	15.97	16.78	16.16	16.71	17.22
SD		0.50	1.11	1.26	0.70	1.19	1.10	1.92	1.68	0.94	1.27	0.93	0.94	0.86	0.93

M - Male; F- Female; SD – Standard deviation;

Table: 22 Toxicity signs observed in Female Rats

Observation*	Days														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Appeared Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Found death	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Catalepsy	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Chromodacryorrhea	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Clonic	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Coma	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Convulsion	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Diarrhea	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Dullness	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Excessive grooming	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Change in Gait	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Hyperactivity	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Lacrimation	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Nasal discharge	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Nasal irritation	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Piloerection	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Polyuria	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Prostration	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Repetitive circling	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Respiratory distress	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Salivation	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Tonic	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Tremor	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Uro-genital staining	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

* No. of animals showing the clinical sign / No. of animals per group

Table:23 Gross Pathology

S. No.	Sex	Animal No.	Status of The animal at the time of receipt at necropsy	Lesions observed at necropsy
1	F	6022	Live	NAD
2	F	6023	Live	NAD
3	F	6024	Live	NAD
4	F	6025	Live	NAD
5	F	6026	Live	NAD

F–Female; NAD- No Abnormalities Detected

Acute oral toxicity studies on aqueous extract of ***Nilavembu Kudineer*** showed no mortality in rats at doses up to 2 g/kg. There were no toxicity signs observed on the skin, fur or eyes of the animals.

From the present study, it is concluded that the ***Nilavembu Kudineer*** is non-toxic upto 2000 mg/kg body weight of oral administration and no significant toxic symptoms were observed during the observation period as well as post mortem examination.

Results-Pharmacological activity

Table: 24 Effect of *Nilavembu Kudineer* on blood glucose against STZ induced diabetic rats

Groups	Blood Glucose level (mg/dl)				
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Normal control	75.38±1.39	75.63±0.53	81.25±1.40	78.25±1.32	58.13±1.47
Diseased control	489.30±40.09z	482.90±44.82z	494.30±32.24z	511.90±26.90z	421.10±27.72z
Metformin (100 mg/kg, p.o)	453.30±27.93z	402.50±18.07z	370.90±15.36z,c	336.50±15.94z,c	244.50±8.43c,z
Nilavembu Kudineer (100 mg/kg, p.o)	492.60±10.17z	425.70±21.83z	438.00±18.87z	426.70±13.43a,z	314.70±23.86b,z
Nilavembu Kudineer (200 mg/kg, p.o)	426.70±37.53z	380.80±20.14z	380.00±20.84b,z	337.00±13.46c,z	279.00±11.75c,z
Nilavembu Kudineer (400 mg/kg, p.o)	464.00±25.23z	417.40±23.15z	353.90±17.72c,z	332.60±15.33c,z	274.40±17.63c,z

Values represented as mean±SEM, n=6, $^{z}P<0.001$, $^{y}P<0.01$, $^{x}P<0.05$ compared with normal control, $^{c}P<0.001$, $^{b}P<0.01$, $^{a}P<0.05$ compared with diseased control,

The anti diabetic effect of ***Nilavembu Kudineer*** evaluated at different dose levels 100, 200 and 400 mg/kg and compared using a positive control in STZ induced hyperglycemia rats are presented in the table: 24. Hyperglycemia was induced by single intrapertoneal injection of streptozotocin and significant ($P<0.001$) increase in blood glucose level was noticed after 48 hours in all rats induced with single injection. The diabetic rats were administered with standard drug (Metformin 100 mg/kg) and three different doses of ***Nilavembu Kudineer*** (100, 200 and 400 mg/kg) were administered orally for a period of 28 days.

Metformin at the dose 100 mg/kg showed significant ($P<0.001$) decrease in blood glucose level on Day 14 of the drug treatment, where as ***Nilavembu Kudineer*** at the dose level 200 and 400 mg/kg also showed significant ($P<0.05$) decrease in glucose levels in STZ induced diabetic rats. The extract at low dose (100 mg/kg) did not produce any significant ($P>0.05$) decrease in blood glucose level in diabetic rats.

On Day 21, diabetic rats treated with low dose of ***Nilavembu Kudineer*** (100 mg/kg) also showed significant ($P>0.05$) decrease in blood glucose level as compared with disease control.

Table: 25 Effect of *Nilavembu Kudineer* on serum biochemical parameters in Diabetic rats

Groups	Glucose (mg/dl)	TGL (mg/dl)	Total Cholesterol (mg/dl)	Urea (mg/dl)	Creati-nine (mg/dl)	Total protein (g/dl)	Albu-min (g/dl)
Normal control	71.00 ±4.62	32.86 ±2.79	60.50 ±3.59	39.98 ±1.78	0.48 ±0.01	5.90 ±0.10	2.77 ±0.06
Diseased control	404.00 ±33.76c	68.50 ±14.52a	51.75 ±2.85	68.73 ±5.32c	0.53 ±0.01a	5.57 ±0.07	2.72 ±0.11
Metformin (100 mg/kg, p.o)	163.50 ±26.04z	43.50 ±2.60	52.00 ±3.84	50.76 ±2.61	0.51 ±0.01	5.75 ±0.19	2.49 ±0.14
Nilavembu kudineer (100 mg/kg, p.o)	428.90 ±33.77	69.57 ±10.25	55.75 ±4.59	59.76 ±6.69	0.50 ±0.01	5.94 ±0.15	2.61 ±0.12
Nilavembu kudineer (200 mg/kg, p.o)	255.80 ±53.55x	70.88 ±6.13	57.63 ±3.23	46.28 ±3.65x	0.46 ±0.01y	5.93 ±0.17	2.91 ±0.14
Nilavembu kudineer (400 mg/kg, p.o)	143.00 ±19.93z	66.00 ±6.50	60.14 ±4.18	45.51 ±4.80y	0.45 ±0.02z	6.17 ±0.07x	2.75 ±0.15

Values represented as mean±SEM, n=6, $^{c}P<0.001$, $^{b}P<0.01$, $^{a}P<0.05$ compared with normal control, $^{z}P<0.001$, $^{y}P<0.01$, $^{x}P<0.05$ compared with diseased control,

After 28 days of drug treatment, all the fasted rats were subjected to blood collection; the serum separated was subjected to biochemical analysis. Serum glucose level in STZ induced rats showed significant (P<0.001) increase when compared with normal control rats. The diabetic rats treated with metformin at the dose level 100 mg/kg for 28 days revealed significant (P<0.001) decrease in serum glucose levels. Whereas 200 and 400 mg/kg ***Nilavembu Kudineer*** treated diabetic rats showed significant (P<0.001) decrease in serum glucose level.

In STZ induced diabetic rats, a significant ($P<0.05$) increase in serum triglycerides levels was observed (68.50±14.52) when compared to normal (32.86±2.79). The diabetic rats treated with reference drug, metformin showed decrease trend in serum triglycerides when compared with STZ induced diabetic rats. Similar trend was also observed in diabetic rats treated orally with ***Nilavembu Kudineer*** at the dose level 400 mg/kg.

Serum urea level in STZ induced diabetic rats showed significant ($P<0.001$) increase compared with normal control rats. The diabetic rats treated with ***Nilavembu Kudineer*** at the dose levels 200 and 400 mg/kg revealed significant ($P<0.05$) decrease in serum urea level compared with STZ induced diabetic rats. Similarly, Serum creatinine level in STZ induced diabetic rats showed significant ($P<0.05$) increase compared with normal control rats. The diabetic rats treated with ***Nilavembu Kudineer*** at the dose levels 200 and 400 mg/kg revealed significant ($P<0.01$) decrease in serum creatinine level compared with STZ induced diabetic rats.

Table: 26 Effect of *Nilavembu Kudineer* on body weight in STZ induced diabetic rats

Groups	Weekly body weight (Grams)					
	Before STZ induction	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Normal control	178.4 ±5.09	195 ±4.97	212.9 ±4.96	220.5 ±5.36	225.5 ±5.56	220.7 ±6.09
Diseased control	182.7 ±8.28	175.3 ±7.97	158 ±9.54	162.8 ±9.18	156.5 ±10.87	157.3 ±11.16
Metformin (100 mg/kg, p.o)	175.3 ±7.79	168.9 ±8.67	151.6 ±8.25	155.4 ±7.97	161.4 ±7.06	154.8 ±8.60
Nilavembu Kudineer (100 mg/kg, p.o)	178 ±4.18	170 ±4.66	155.4 ±7.17	156.2 ±9.88	172 ±8.89	159.3 ±9.28
Nilavembu Kudineer (200 mg/kg, p.o)	185.7 ±4.28	178.1 ±4.04	161.3 ±6.30	163.6 ±7.92	164.9 ±8.17	161.8 ±8.50
Nilavembu Kudineer (400 mg/kg, p.o)	176.7 ±4.77	168.4 ±6.73	151.7 ±10.39	155.9 ±10.58	154.3 ±11.89	145.7 ±12.79

Values represented as mean±SEM, n=6

The weekly body weight changes of different groups of rats are given in table 5. It was observed that there were no significant changes in body weight in diabetic rats treated with ***Nilavembu Kudineer*** when compared with STZ treated rats throughout the entire period of drug treatment.

Table: 27 Effect of *Nilavembu Kudineer* on feed and water intake and urine output in STZ induced diabetic rats

Groups	**Feed intake (g/rat/day)**	**Water intake (ml/rat/day)**	**Urine output (ml/rat/day)**
Normal control	26.1±2.4	38.3±1.7	10.3±0.3
Diseased control	74.3±4.1c	126.7±12.0c	113.3±8.8c
Metformin (100 mg/kg, p.o)	42.8±4.3y	120.0±5.8	86.7±8.8
Nilavembu Kudineer (100 mg/kg, p.o)	56.9±4.5	126.7±6.7	98.3±10.9
Nilavembu Kudineer (200 mg/kg, p.o)	50.6±2.4x	111.7±10.1	75.0±8.7x
Nilavembu Kudineer (400 mg/kg, p.o)	46.5±5.0y	88.3±6.0x	48.3±6.0y

Values represented as mean±SEM, n=3, $^{c}P<0.001$, $^{b}P<0.01$, $^{a}P<0.05$ compared with normal control, $^{z}P<0.001$, $^{y}P<0.01$, $^{x}P<0.05$ compared with diseased control

Feed intake, water intake and urine output were measured using metabolic cages and the values were tabulated. Feed intake, water intake and urine output was measured during 4th week of drug administration in STZ induced diabetic rats. All the diabetic rats showed significant (P<0.001) increase in feed intake, water intake and urine output when compared with normal rats. Diabetic rats treated with extract for 28 days were able to produce significant (P<0.001) decrease in water consumption and urine output, when compared with STZ induced diabetic rats.

Histopathological findings of liver, Kidney and pancreas in *Nilavembu Kudineer* treated diabetic rats are photo-micrographed and presented in sequel.

LIVER

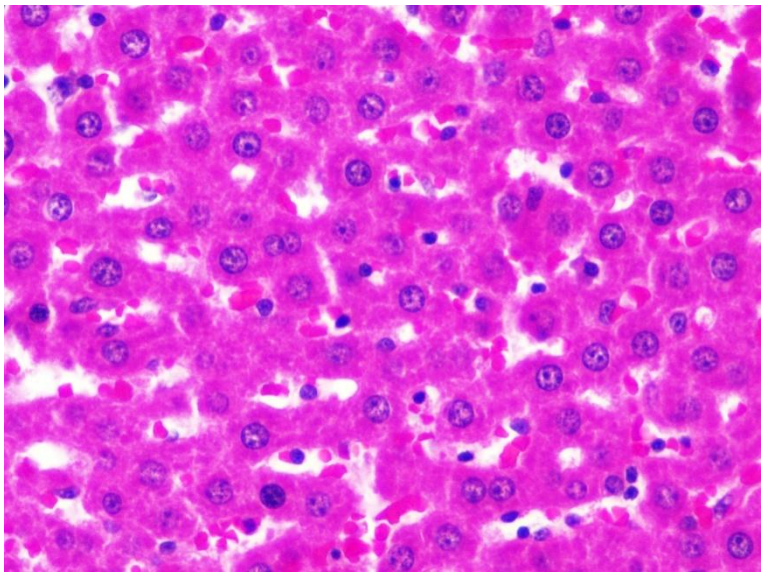


Fig-12 **CONTROL**

Within normal limits

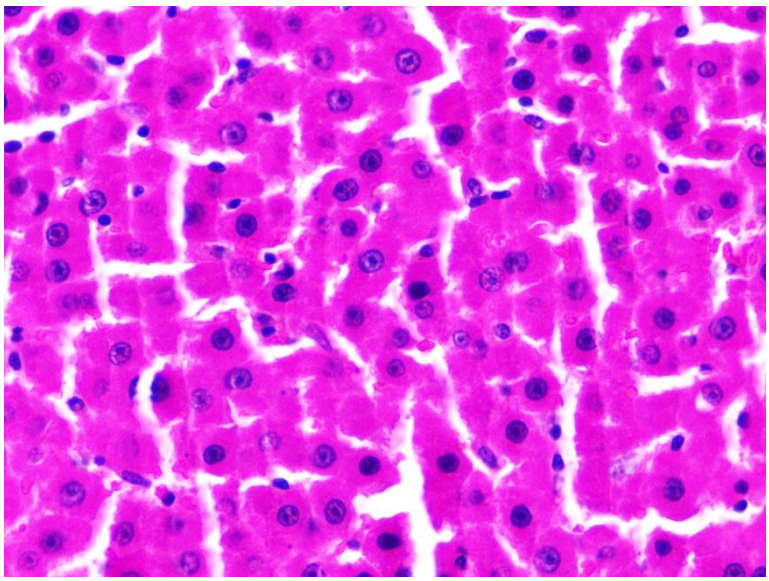


Fig-13 **DISEASE CONTROL**

Infiltrates, mononuclear, peribiliary/parenchymal, diffuse, mild
Micrograniuloma, multiple, random, minimal
Necrosis, hepatocellular, multifocal, random, minimal

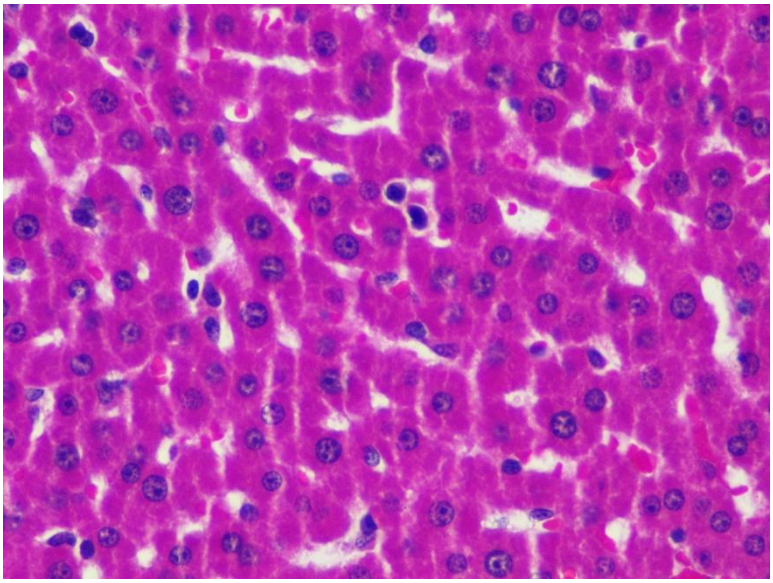


Fig-14 **STANDARD**
Atrophy, hepatocellular, diffuse, minimal

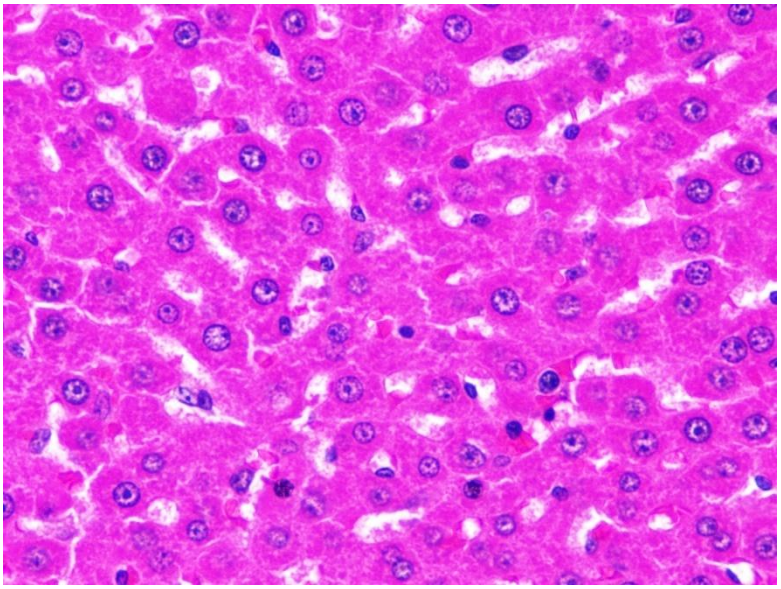


Fig-15 **LOW DOSE**
Infiltrates, mononuclear, peribiliary/parenchymal, diffuse, mild

Microgranuloma, multiple, random, mild

Necrosis, hepatocellular, multifocal, random, mild

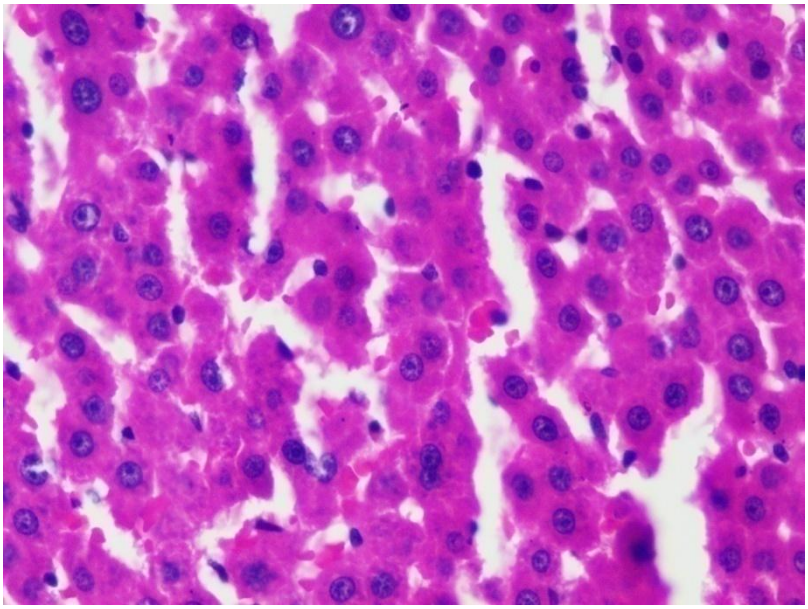


Fig-16 **MEDIUM DOSE**
Within normal limits

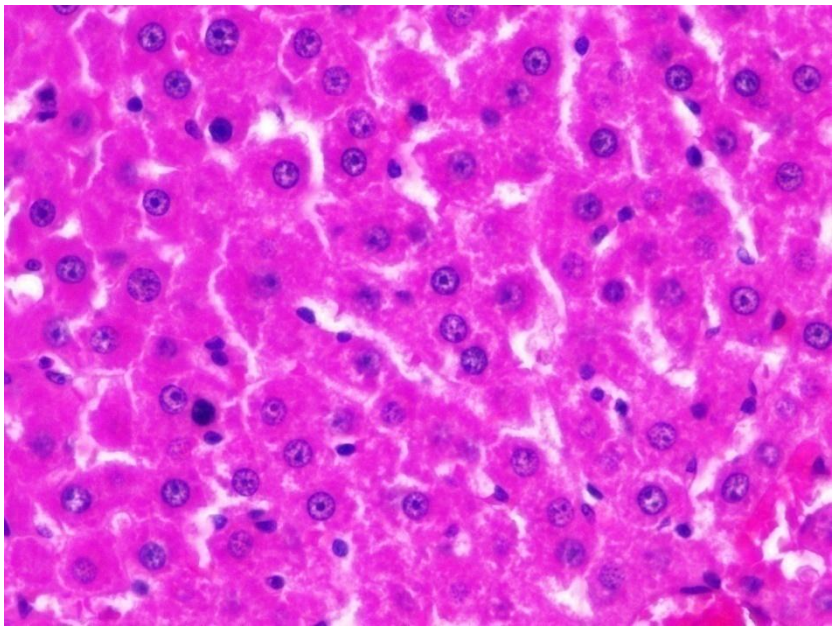


Fig-17 **HIGH DOSE**
Infiltrates, mononuclear, peribiliary/parenchymal, diffuse, minimal

Microgranuloma, multiple, random, minimal

Necrosis, hepatocellular, multifocal, random, minimal

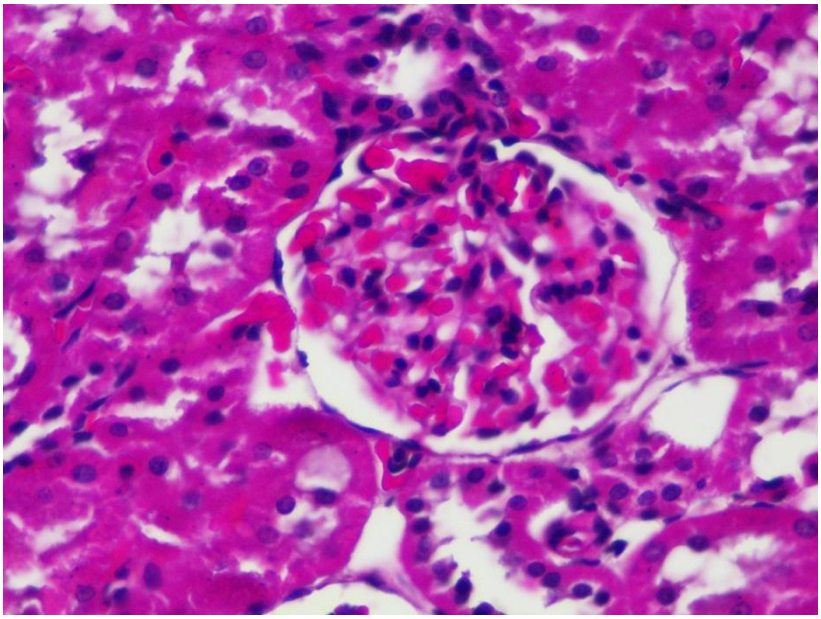


Fig-18 CONTROL (Kidney)

Infiltrates, mononuclear, interstitial, multifocal, minimal

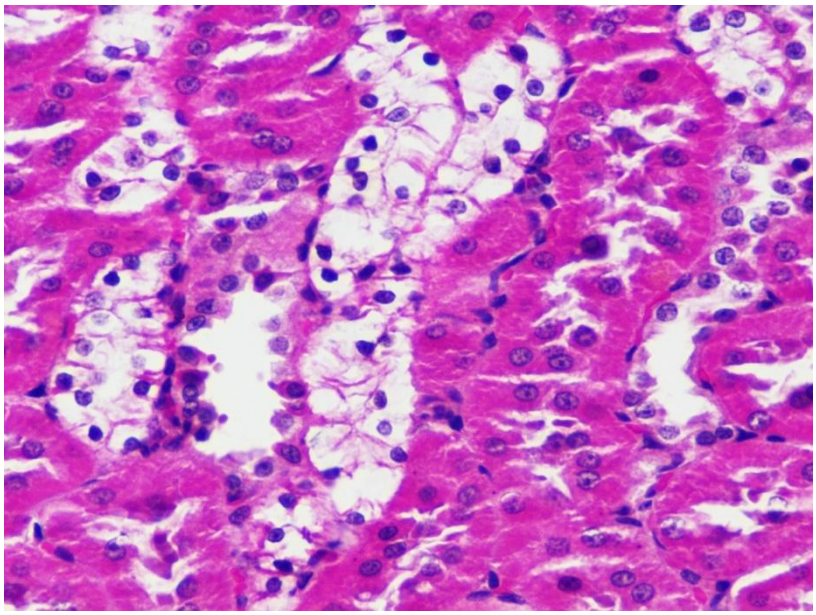


Fig-19 DISEASE CONTROL(Kidney)

Vacuolation/ degeneration, tubular, diffuse, mild

Infiltrates, mononuclear, interstitial, multifocal, mild

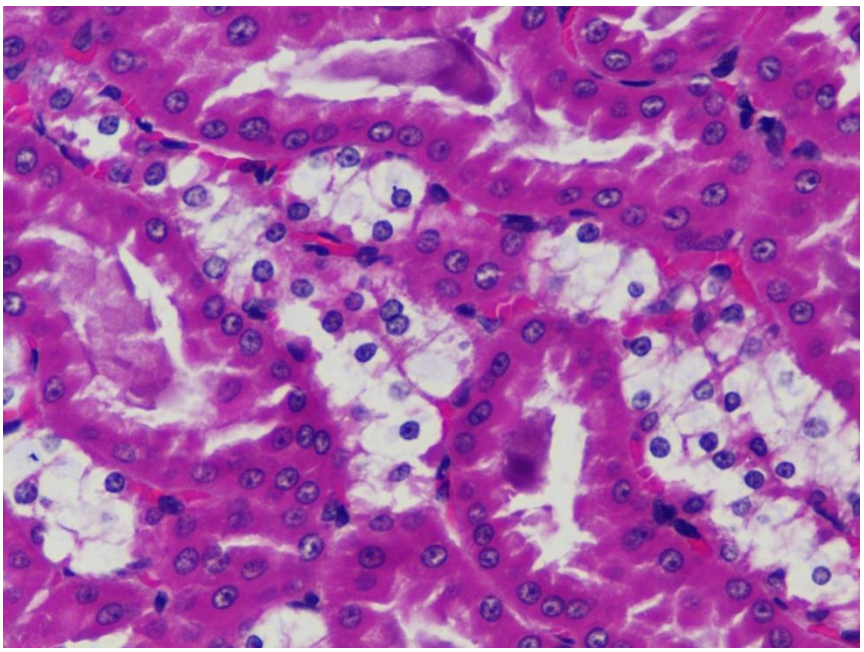


Fig-20 STANDARD(Kidney)

Vacuolation/ degeneration, tubular, diffuse, mild

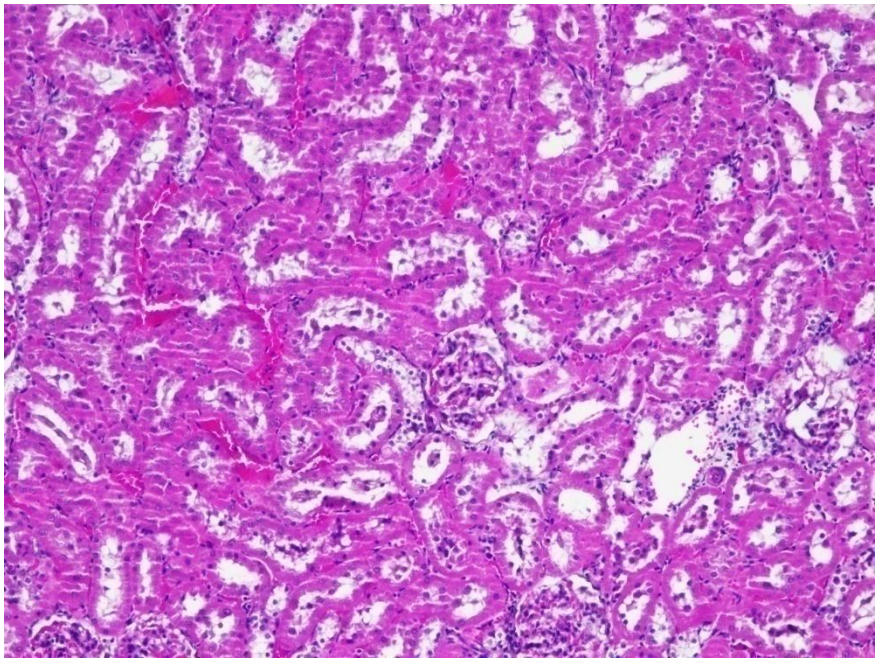


Fig-21 LOW DOSE(Kidney)

Vacuolation/ degeneration, tubular, diffuse, minimal

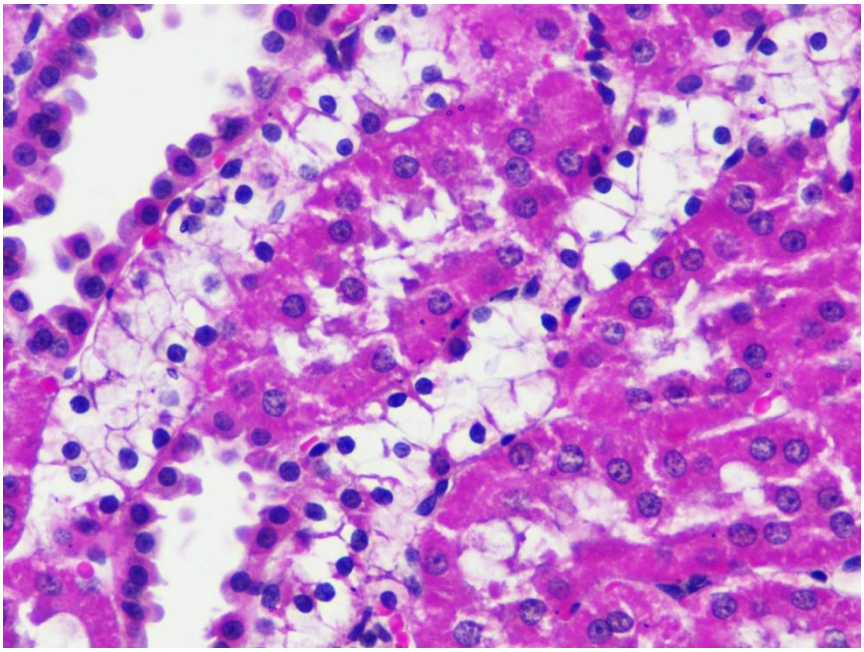


Fig-22 MEDIUM DOSE(Kidney)

Vacuolation/ degeneration, tubular, diffuse, mild

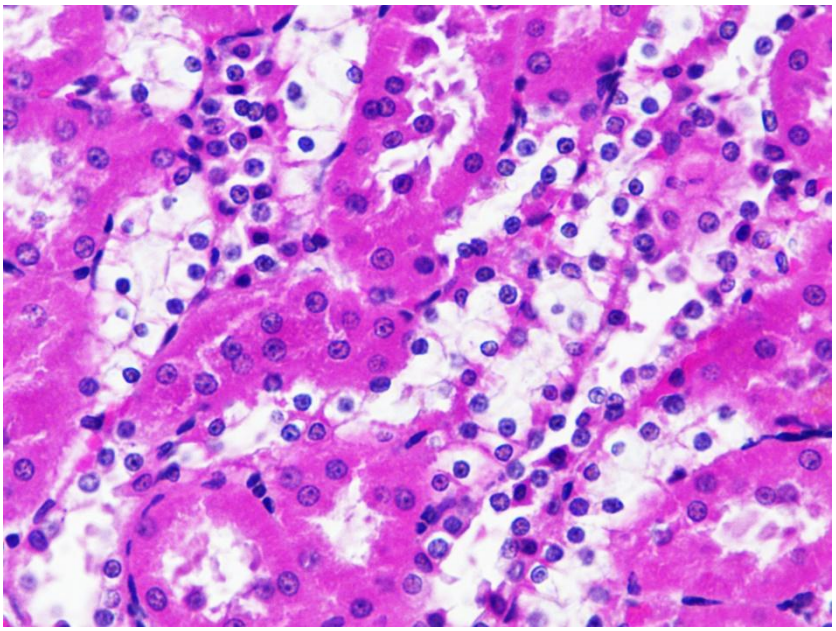


Fig-23 HIGH DOSE(Kidney)

Vacuolation/ degeneration, tubular, diffuse, mild

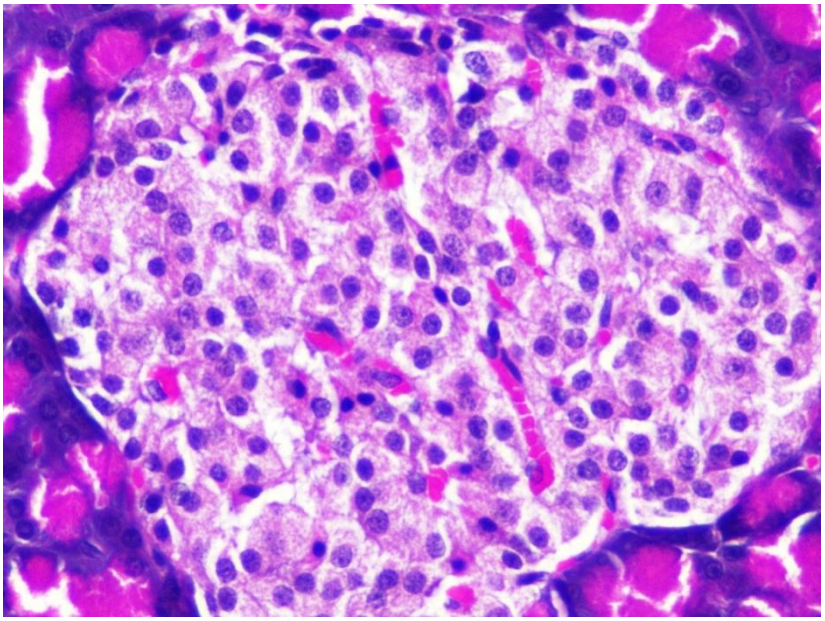


Fig-24 **CONTROL (Pancreas)**
Within normal limits

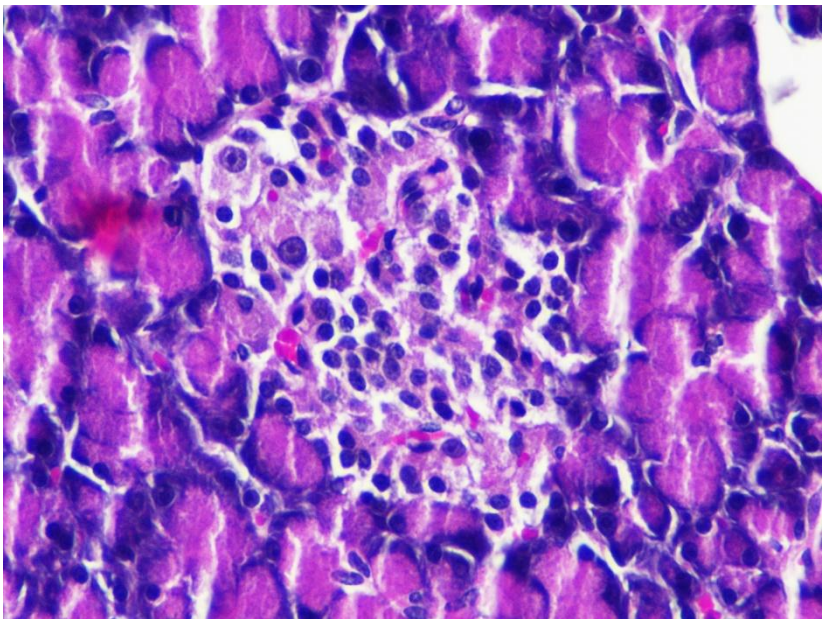


Fig-25 **DISEASE CONTROL(Pancreas)**

Atrophy / degeneration, Islet cells, diffuse, marked

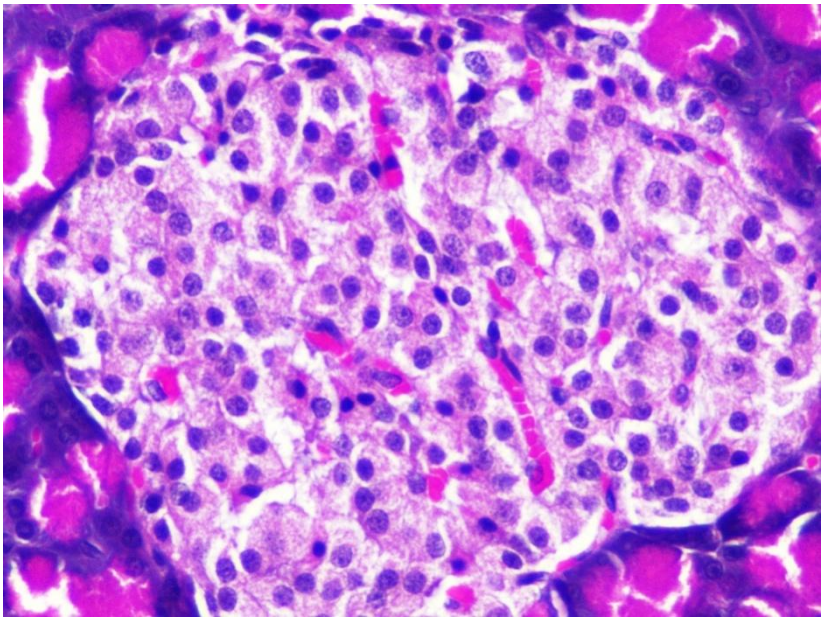


Fig-26 **STANDARD(Pancreas)**

Atrophy / degeneration, Islet cells, diffuse, minimal

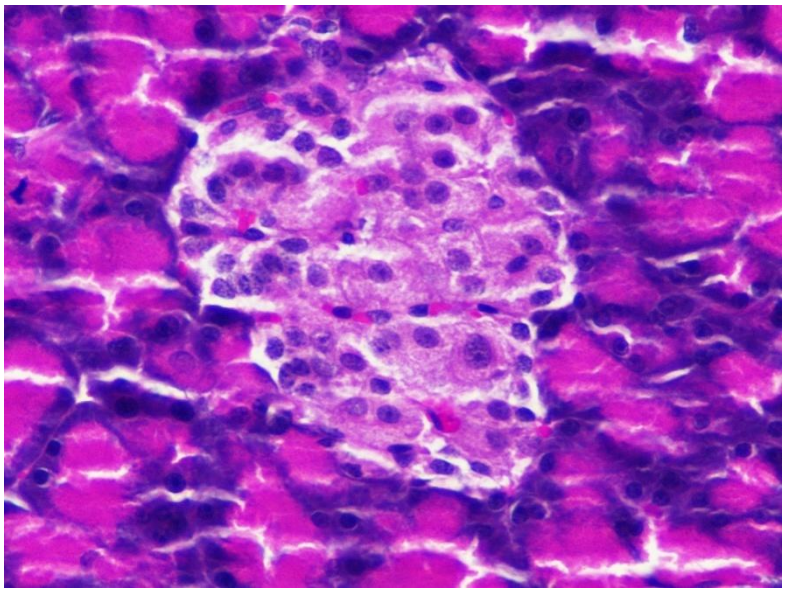


Fig-27 **LOW DOSE(Pancreas)**

Atrophy / degeneration, Islet cells, diffuse, minimal

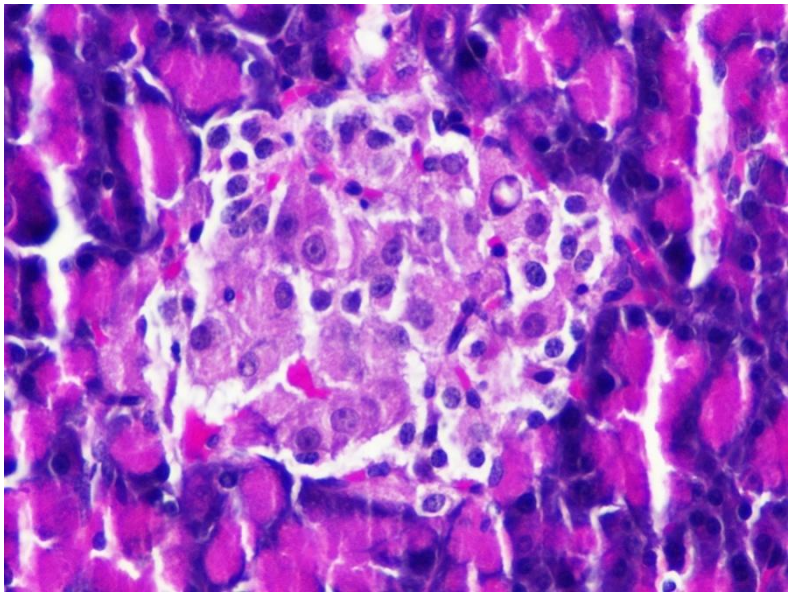


Fig-28 **MEDIUM DOSE(Pancreas)**

Atrophy / degeneration, Islet cells, diffuse, mild

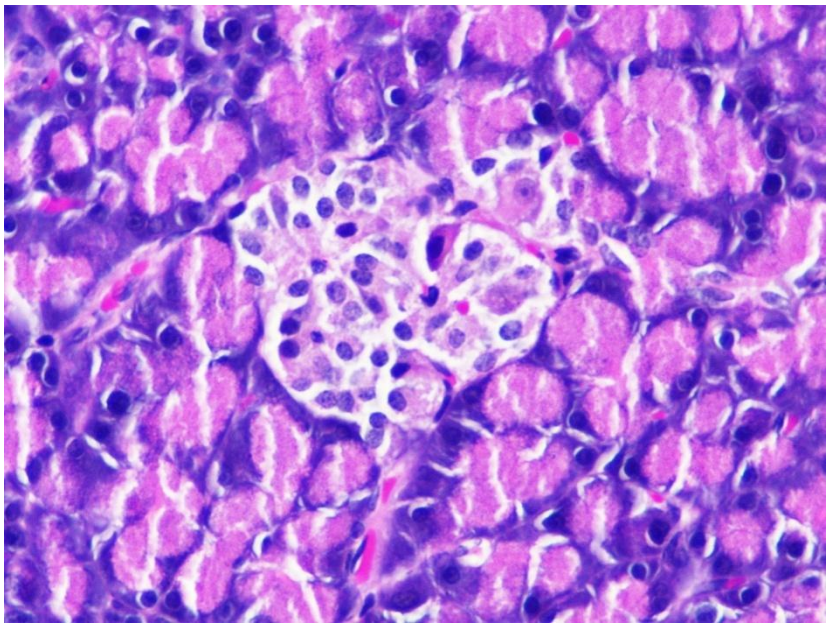


Fig-29 **HIGH DOSE(Pancreas)**

Atrophy / degeneration, Islet cells, diffuse, mild

From the photomicrographs of histopathological studies, it is inferred that altered cellular architecture observed in the liver, kidney and pancreas of diabetic rats were brought back to near normalcy on administration of ***Nilavembu Kudineer***.

6.13 Clinical trial studies

- Total number of patients screened 90
- No. of Patients selected for the study 60
- No. of dropped out 12
- No. of cases participated throughout the study period 48

Table: 28 Socio-demographic Features

Diabetic Studies		**Male**	**Female**
No. of Cases		26	22
Age		30-44	30-59
Locality	Rural	16	10
	Urban	12	10
Dietary Habit	Veg.	6	8
	Mixed Diet.	20	14
Smoking/Tobacco Consumption		8	5
Economic Status	Low	7	5
	Middle	12	9
	High	7	8
Educational Status	Literate	21	14
	Illiterate	5	8

Table:29 Naa (Tongue) observations

Observations	Before Treatment	After Treatment
Pale	22	18
Coating	10	8
Dryness	21	20
Redness	3	2
Fissures in margin	0	0
Alteration in taste	15	10
Alteration in teeth and gums	20	17
Normal	20	20

Table: 30 Body Constitutions

S. No	Constitution of the body	No. of cases
1.	Vatha Thegi	10
2.	Pitha Thegi	22
3.	Kapha Thegi	6
4.	Thontha Thegi	10

Table:31 Distribution of Season

S. No	Season	No. of cases
1.	Kaar Kaalam	6
2.	Koothir Kaalam	3
3.	Munpani Kaalam	15
4.	Pinpani kaalam	8
5.	Ilaveni Kaalam	10
6.	Muthuveni Kaalam	6

Table:32 Distribution of Uyir Thathukkal

Uyir Thathukkal	Before treatment	After treatment
Vatham		
Pranan	22	17
Abanan	38	26
Viyanan	22	18
Udhanan	20	15
Samanan	17	12
Nagan	9	7
Koorman	8	6
Kirukaran	5	3
Devathathan	22	11
Dhananjeyan	15	12
Pitham		
Anarpitham	26	23
Ranjaga Pitham	15	12
Prsaka Pitham	18	14
Alosaga Pitham	19	15
Sathaga Pitham	22	11
Kabham		
Avalam bagam	10	23
Kilethagam	22	12
Pothagam	28	12
Tharpagam	12	11
Santhigam	28	21

Table: 33 Distribution of Status of Envagai Thervugal

Envagai Thervu	Affected Thathu	Before treatment	After treatment
Naa	Vatha kuttram	12	10
	Pithakuttram	22	21
	Kabhakuttram	4	2
	Iyalbu(normal)	10	10
Niram	Vatha kuttram	5	2
	Pithakuttram	6	5
	Kabhakuttram	3	2
	Iyalbu(normal)	34	34
Mozhi	Vatha kuttram	4	2
	Pithakuttram	5	4
	Kabhakuttram	2	1
	Iyalbu(normal)	37	37
Vizhi	Vatha kuttram	11	9
	Pithakuttram	18	14
	Kabhakuttram	5	3
	Iyalbu(normal)	14	14
Sparism	Vatha kuttram	4	3
	Pithakuttram	6	3
	Kabhakuttram	2	1
	Iyalbu(normal)	36	36
Moothiram	Vatha kuttram	12	8
	Pithakuttram	24	18
	Kabhakuttram	8	6
	Iyalbu(normal)	4	4
Malam	Vatha kuttram	12	10
	Pithakuttram	22	17
	Kabhakuttram	8	4
	Iyalbu(normal)	4	3
Naddi	Vatha kuttram	8	6
	Pithakuttram	24	20
	Kabhakuttram	6	3
	Iyalbu(normal)	10	10

Table: 34 Involvement of Ezhu Udalkattugal (Seven Body Constituents)

S. No	Udalkattugal	No. of cases	percentage
1.	Saaram – Serum	30	62
2.	Senneer – Blood	48	100
3.	Oon – Muscle	22	46
4.	Kozhuppu – Fat	12	27
5.	Enbu – Bone	2	4.6
6	Moolai – Brain	2	4
7.	Sukkilam/Suronitham – Semen/ovum	5	11

Table:35 Evaluation of effect of *Nilavembu Kudineer* on Hemopoetic function

Parameters	Normal Range	Before Treatment	After Treatment
Lymphocytes (%)	20-45	29.72 ±4.28	28.82 ±4.73
Eosinophils (%)	01-06	5.42 ±1.24	4.29 ±1.37
Platelet count (lacs/cumm)	1.5-4.0	2.38 ±0.63	2.55 ±0.47
Hb (g/dL)	M-12-17 F 12-15	12.9±1.89	12.9±1.48

Table:36 Evaluation of effect of *Nilavembu Kudineer* on Liver function

Parameters	Normal Range	Before Treatment	After Treatment
Serum billirubin (mg/dl)	0.3-1.0	0.63 ±0.18	0.60 ±0.18
Total protein (gm /dl)	6.0-8.5	6.52 ±1.26	6.60 ±1.36
Albumin (gm/dl)	3.5-5.0	3.69 ±1.38	3.40 ±1.30

Table:37 ***Data on Renal function test following Nilavembu Kudineer therapy***

Factors	**Normal Range**	**Before Treatment**	**After Treatment**
Blood Urea (mg/dL)	10-50	24±3.95	23.5±3.52
Serum Creatinine (mg/dL)	M 0.9-1.3 F 0.6-1.1	0.77±0.15	0.77±0.25

Table:38 Effect of *Nilavembu Kudineer* on Lipids

Treatment group	**Normal Range**	**Before Treatment**	**After Treatment**
Triglycerides	< 170 mg/dL	134±29.7	137±30.9
Serum Cholesterol	Upto 200 mg/Dl	178±22.1	176±21.4

Table:39 Effect of *Nilavembu Kudineer* on Fasting and Post Prandial Blood Glucose

Treatment group	**Normal Range**	**0 Day**	**30 days**	**45days**	**60 days**	**90 days**
Fasting Blood Glucose (mg/dL)	70 – 110	129 ± 11.7	126 ± 11.3	124 ± 12.3	123 ± 12.3	122 ± 10.8
Post prandial Blood Glucose (mg/dL)	80 – 140	221± 22.3	219 ± 25.0	215 ± 27.0	214 ± 27.0	208 ± 27.7
HbA1c (%)	< 6.5 %	8.35±0.62	-	-	-	7.75±0.90

Findings

The diagnosis and prognosis was done on the basis of all the above mentioned Siddha and modern parameters and the results are summarized below.

Out of the 60 subjects selected, 15 cases dropped out in the middle of the study and only 48 (22 females, 26 males) cases cooperated till the end of the study.

Clinical observations

- 6 subjects showed *vatha naadi*, 38 subjects showed *pithta naadi* and 4 subjects showed *kapha* and *thontha naadi*.
- Based on body proportion 22 pitha subjects, 16 kapha subjects and 10 vatha subjects were identified. Among this, pitha and kapha people showed marked improvement whereas vaatha people showed only symptomatic improvement
- Disorientation of pitha was found in 38 patients out of which 28 recovered. 2 of the 4 subjects with kapha disorientation, and 3 out of 6 subjects with vatha disorientation also recovered to normal. This indicates that this plant was more effective in treating pitha dominated diabetic complications as compared to vatha and kapha dominated complications.
- Significant changes in fasting blood sugar were noted only in 28 patients
- But 25 subjects showed significant improvement in post prandial glycemic level after treatment.
- Glycated hemoglobin markedly reduced in 20 patients
- Most of the subjects reported reduction in excessive appetite, which is the most common symptom of Type II Diabetes.

CHAPTER – VII
DISCUSSION

7.1 DISCUSSION

In the past decades, diabetes mellitus has become a major health problem world wide, reaching epidemic proportions in many developing countries including India. **World wide projections** suggest that >220 million people will have diabetes by the year 2020 and majority of these (approximately 213 million) will have type-II diabetes. Recently Diabetes mellitus has emerged as a leading metabolic disorder. India is the diabetic capital of the world, with 41 million people affected with the disease.

In spite of greater advancements made in understanding and managing this disease, the graph of diabetes-related complications and mortality are rising unabatedly. With an objective of identifying a safe and novel anti-diabetic drug from traditional medicinal sources, Siddha drug ***Nilavembu Kudineer*** is selected for the present study and evaluated for its anti-diabetic and toxicity profiles employing Streptozotocin (STZ) induced diabetic rats.

The selected drug is also assessed for its clinical efficacy in Type 2 diabetes mellitus patients. Besides the selected Siddha herbal formulation is also studied from chemical and botanical standardization point of view in order to contribute to the suffering diabetic population **a quality standard anti diabetic Siddha herbal drug.**

7.2 Botanical studies: Powder microscopic analysis of the preparation

Powder microscopy of ***Nilavembu Kudineer*** revealed the presence of all the ingredients mentioned in the Siddha text. The unique microscopic features of the ingredients of ***Nilavembu Kudineer*** were compared with the microscopic characters which are mentioned in the Siddha Pharmacopoeia and other literatures were compared with given curnam.

7.2.1Botanical standards determined

Based on the powder microscopy following botanical standards were determined for the ***Nilavembu Kudineer***:

- Wavy epidermal cells
- Sclerenchyma cells with narrow lumen
- Vessels with simple pits and scalariform thickening, parenchyma cells with reticulation
- Tracheids are thick walled
- Fibres with branching and split ends, libriform, thin walled, with tapering end wide lumen
- Polygonal cork cells
- Presence of tricolpate pollen grains and group of fibres and brown content, slightly elongated and beaker shaped stone cells
- Presence of unicellular and short and long uniseriate trichomes with bi and tri cellular, short and long uniseriate multicellular headed glandular trichome
- Presence of stone cells and various shaped and striated sclereids
- Starch grains round to oval calcium oxalate crystals prismatic and acicular

7.3 Chemical standardization studies:

Physico chemical constants were determined as per the standard siddha pharmacopoeia

Chemical standards

Based on the studies following chemical standards for identity, purity and strength were determined for ***Nilavembu Kudineer***.

Test for identity, purity and strength

- pH not more than 7
- Loss on drying not more than 6%
- Total ash not more than 31%
- Acid insoluble ash not more than 2%
- Alcohol soluble extractives not less than 4%
- Water soluble extractives not less than 81%

Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of various bioactive compounds like alkaloids, sterols, terpenoids, saponins, carbohydrates and mucilage in ***Nilavembu Kudineer***. The tests for flavonoids, tannins and phenols gave negative results, which indicated their absence in ***Nilavembu Kudineer***.

7.4 HPTLC Finger printing studies

The HPTLC analysis of ***Nilavembu Kudineer*** revealed the presence of 11 spots with R_f at 0.31, 0.55, 0.58, 0.65, 0.70, 0.77, 0.82, 0.88, 0.92, 0.95 and 0.97 under 254 and 366 nm. The R_f value 0.70 indicates the presence of Andrographolide, the marker compound.

7.5 GC-MS studies

The GC-MS analysis of ***Nilavembu Kudineer*** showed the presence of vetiveric acid, palmitic acid, gingerol, santalol, piperine and andrographolide in the selected formulation suggesting the presence of few ingredients such as Andrographis, Vettiver, Ginger, Santalumalbum and Pepper. Some of the anti-diabetic molecules such as vettiveric acid, piperine and andrographolide were also identified in the GC-MS analysis.

FTIR analysis of ***Nilavembu Kudineer*** revealed the presence of alkanes, amine, carboxylic, hydroxyl and aromatic functional groups.

7.6 In vitro cytotoxicity

The aqueous extract was prepared by taking 25 g of ***Nilavembu Kudineer*** raw material in 500 ml of distilled water and boiled at 90°C until the final volume reaches to 100 ml. Then the content was filtered and the filtrate was frozen and then lyophilized. The extract yield was found to be 1.32 g/100 g drug. The extract was re-suspended in water at 10 mg/ml ratio and used for the cytotoxicity experiment. For evaluating cytotoxicity 3T3-L1 pre-adipocytes were obtained from the National Center for Cell Science (NCCS), Pune and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and penicillin (100 U/mL)-streptomycin (100 μg/mL) in a humidified atmosphere of 95% air and 5% CO2 at 37°C. After arriving 60% of confluency, the cells were trypsinized and dispersed in a 96 well plate with a cell count of 9000 cells per well and incubated for 24 h. Then the aqueous extract of the selected formulation was added at

different concentrations (10, 5, 2.5, 1.25 and 0.625 mg/ml) and then again incubated for 24 h. The cell group grown in medium without plant extract was considered as control. At the end, the medium was discarded, cells are washed with PBS and then MTT reagent (20 µL) was added in each well and incubated for 6 hrs at 37°C in a water bath. Then 150 µL of acidic isopropanol was added and shaken for 30 min on a plate shaker under dark. The absorbance was measured at 540 nm in a micro-plate reader and the cell viability was calculated and expressed in percentage basis. Based on the data obtained in the present study, it is concluded that the aqueous extract of ***Nilavembu kudineer*** is not toxic at a concentration range of 0.625 – 10 mg/ml in cell line model.

7.7 Acute oral toxicity

Acute oral toxicity study was performed for aqueous extract of ***Nilavembu Kudineer*** as per OECD 425 guideline (OECD, 2008). All procedures involving laboratory animal use were in accordance with the Institute Animal Ethics Committee regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the approval number is 357/SASTRA/IAEC/RPP. Healthy Female Albino Wistar rats were selected for the studies and administered with a single oral dose of aqueous extract of ***Nilavembu Kudineer*** at the dose of 2000 mg/kg, b.wt by oral route dissolved in 1 mL of distilled water used as the vehicle. Each animal was observed for every 15 min in the first 4 h after dosing, then every 30 min for the successive 6 h and then daily for the successive 48 h for the short-term outcome and the remaining 14 days for the long-term possible lethal outcome which in this case was death. The animals were observed for signs of convulsions, tremors, circling, depression, excitement and mortality. The surviving animals were sacrificed under CO_2 inhalation euthanasia, autopsied and examined macroscopically for any pathological changes. Acute oral toxicity studies of aqueous extract of ***Nilavembu Kudineer*** showed no mortality in rats at doses up to 2 g/kg. There were no toxicity signs observed on the skin, fur or eyes of the animals. No noticeable behavioral changes in salivation, sleeping pattern, diarrhea or lethargy were observed in the treated animals. These results indicate that aqueous extract of ***Nilavembu Kudineer*** is non-toxic, and safe up to 2 g/kg, b. wt. through oral route.

7.7.1Anti-diabetic activity

Anti-diabetic screening of aqueous extract of ***Nilavembu Kudineer*** against Streptozotocin (STZ)-Induced Diabetics in Rats was conducted. Male Albino Wistar rats were used for screening anti-diabetic potential of the selected formulation. Diabetes was induced by a single intraperitoneal injection of Streptozotocin (STZ, 65 mg/kg) dissolved in 0.1 M citrate buffer (pH 4.5) in fasted rats. The control group rats were administered with distilled water. The Diabetes was confirmed at 48 h after STZ injection by measuring the glucose concentrations of peripheral blood obtained from the tail vein using glucometer. Rats with blood glucose levels of 250 mg/dl or higher were considered to be diabetic and were grouped into six groups each containing eight rats. The diabetic induced rats were treated with vehicle/standard/test drug orally as per the study protocol for 4 weeks. The change in body weights and blood glucose level were measured weekly. During the last week of drug treatment, experimental animals were housed in metabolic cages and the changes in feed intake, water intake and urine output were measured and calculated. At the end of the experiment, all the rats were fasted overnight and blood samples were collected by retro orbital puncture under mild anesthesia. The blood samples were collected in serum activator tubes and centrifuged at 1000 g for 15 min to obtain serum. The serum layer was collected in properly labeled, clean and micro-centrifuge tubes and analyzed immediately for biochemical parameters such as total protein, total cholesterol and triglycerides levels. For histopathological study, pancreas, liver and kidney tissues were collected and fixed in 10% buffered formalin solution. After a minimum of 24 hour fixation, the samples were stained using Hematoxylin and eosin. They were examined under a light and polarized microscope. All deviations from normal histology were recorded and compared with the corresponding controls.

Streptozotocin (STZ) is widely used experimentally to induce diabetes in animals (Zusman *et al*., 1985). STZ after uptake into pancreatic beta cells, is split up into glucose and methylnitrourea moiety. The latter, due to its alkylating properties, modifies biological macromolecules, fragments of DNA and destroys the beta cells thus causing diabetes (Szkudelski 2001, Okamoto,1981).

The major symptoms of diabetes are hyperglycemia along with polyuria, polydipsia and polyphagia. In the present study, STZ induced rats showed three characteristic symptoms such as polyuria, polydipsia and polyphagia. ***Nilavembu Kudineer*** treatment for 28 days has brought back the above mentioned symptoms to normal.

Decreased body weight observed in diabetic control rats in comparison to normal rats indicates that loss of body weight is a result of excessive breakdown of tissue proteins (Salahuddi et al., 2010). Kondeti *et al* have reported that STZ induced diabetic rats account for the observed decrease in the total protein content.

One of the most sensitive and dramatic indicators of kidney injury is to increase the creatinine and urea level in serum. Increased urea production in diabetes might be due to enhanced catabolism of both liver and plasma proteins (Kondeti et al., 2010).

The diabetic hyperglycemia induces elevation of the serum levels of urea and creatinine which were considered as significant markers of renal dysfunction (Almdal and Vilstrup, 1988). In the present study, STZ induced rats showed significant increase in the level of serum urea and creatinine, after treatment with ***Nilavembu Kudineer*** in diabetic rats for 28 days elevated serum urea and creatinine levels were found to be decreased significantly. The stabilization of these renal parameters by ***Nilavembu Kudineer*** suggests its protection against Diabetic nephropathy, which is one of the diabetic complications observed in chronic diabetic patients. If used as hypoglycemic agents, may also reverse dyslipedemia associated with diabetes and prevent the cardiovascular complications which are very prevalent in diabetic patients.

7.8 Clinical Studies

After the approval of Institute Human Ethical Committee, a pilot scale human clinical study was conducted at National Institute of Siddha, Thambaram Sanitorium, Chennai.

In Naadi examination, *pitha vatha naadi* and *vatha pitha naadi* were found in most of the patients, Out of 90 patients screened, 60 patients had the positive family history of Diabetes mellitus, thus it approves the statement that hereditary factors influence the occurrence of Diabetes mellitus.

Selected 60 human subjects belonging to both sexes in the age group between 30 yrs to 60 yrs were examined for clinical symptoms and were treated as Out- Patients and were administered with ***Nilavembu Kudineer*** at the dose level of 60 ml once a day. 12 subjects dropped during the study period 48 subjects continued through out the study period.

7.8.1 Clinical Findings

The diagnosis and prognosis was done on the basis of all the above mentioned Siddha and modern parameters and the results are summarized below.

Out of the 60 subjects selected, 12 cases dropped out in the middle of the study and only 48 (22 females, 26 males) cases cooperated till the end of the study.

7.8.2Clinical observations

- 6 subjects showed *vatha naadi*, 38 subjects showed *pithta naadi* and 4 subjects showed *kapha* and *thontha naadi*.
- Based on body proportion 22 *pitha* subjects, 16 *kapha* subjects and 10 *vatha* subjects were identified. Among this, *pitha* and *kapha* people showed marked improvement whereas *vatha* people showed only symptomatic improvement
- Disorientation of *pitha* was found in 38 patients out of which 28 recovered. 2 of the 4 subjects with *kapha* disorientation, and 3 out of 6 subjects with *vatha* disorientation also recovered to normal. This indicates that this plant was more effective in treating *pitha* dominated diabetic complications as compared to *vatha* and *kapha* dominated complications.
- Significant changes in fasting blood sugar were observed only in 28 patients
- 30 subjects showed significant improvement in post prandial glycemic level after treatment.
- Glycated hemoglobin was markedly reduced in 20 patients
- Most of the subjects reported reduction in excessive appetite, which is the most common symptom of Type II Diabetes.

The data of the results obtained clearly depict the anti-diabetic efficacy of ***Nilavembu Kudineer*** in human subjects.

To conclude the study drug, ***Nilavembu Kudineer*** is proved for its quality, both botanically and chemically and is found to be safe and effective in the management of type 2 Diabetes mellitus.

CHAPTER VIII

Summary and Conclusion

8.1 Summary and Conclusion

Herbs and herbal formulations used in traditional medicines in Siddha have been widely accepted in India as good therapeutical agents with lower side effects particularly in the management of Diabetes mellitus, a metabolic disorder which is growing exponentially at an alarming rate. But there exist lacuna in these traditional medicines such as lack of standards for products as well as the processes their safety and efficacy. Hence the need of the hour is to scientifically validate and conduct studies on quality, safety and efficacy of these traditional herbal formulations. With these objectives, in the present dissertation a traditional Siddha formulation ***Nilavembu Kudineer*** is selected based on the literature survey and scientifically evaluated for its anti-diabetic efficacy through both preclinical and clinical trial studies. Besides the selected Siddha herbal formulation is also studied from chemical and botanical standardization point of view in order to contribute to the suffering diabetic population **a quality standard anti diabetic Siddha herbal drug.**

Following quality standards were determined for ***Nilavembu Kudineer***

Microscopic standards

- Wavy epidermal cells
- Sclerenchyma cells with narrow lumen
- Vessels with simple pits and scalariform thickening, parenchyma cells with reticulation
- Tracheids are thick walled
- Fibres with branching and split ends, libriform, thin walled, with tapering end wide lumen
- Polygonal cork cells
- Presence of tricolpate pollen grains and group of fibres and brown content, slightly elongated and beaker shaped stone cells
- Presence of unicellular and short and long uniseriate trichomes with bi and tri cellular, short and long uniseriate multicellular headed glandular trichome
- Presence of stone cells and various shaped and striated sclereids
- Starch grains round to oval calcium oxalate crystals prismatic and acicular

8.2 Test for identity, purity and strength

- pH not more than 7
- Loss on drying not more than 6%
- Total ash not more than 31%
- Acid insoluble ash not more than 2%
- Alcohol soluble extractives not less than 4%
- Water soluble extractives not less than 81%

Major chemical constituents

Alkaloids, sterols, terpenoids, saponins, carbohydrates and mucilage

8.3 HPTLC profiles

The HPTLC analysis of ***Nilavembu Kudineer*** revealed the presence of 11 spots with R_f at 0.31, 0.55, 0.58, 0.65, 0.70, 0.77, 0.82, 0.88, 0.92, 0.95 and 0.97 under 254 and 366 nm. The R_f value 0.70 indicates the presence of Andrographolide, the marker compound.

8.4 Acute oral toxicity studies

Data of the results indicated that aqueous extract of ***Nilavembu Kudineer*** is non-toxic, and safe up to 2 g/kg, b. wt. through oral route.

Anti-diabetic activity

In the present study, STZ induced rats showed significant increase in the level of serum urea and creatinine, after treatment with ***Nilavembu Kudineer*** in diabetic rats for 28 days elevated serum urea and creatinine levels were found to be decreased significantly. The stabilization of these renal parameters by ***Nilavembu Kudineer*** suggests its protection against Diabetic nephropathy, which is one of the diabetic complications observed in chronic diabetic patients. If used as hypoglycemic agents, may also reverse dyslipedemia associated with diabetes and prevent the cardiovascular complications which are very prevalent in diabetic patients.

8.5 Clinical Studies

- 6 subjects showed *vatha naadi*, 38 subjects showed *pithta naadi* and 4 subjects showed *kapha* and *thontha naadi*.
- Based on body proportion 22 *pitha* subjects, 16 *kapha* subjects and 10 *vatha* subjects were identified. Among this, *pitha* and *kapha* people showed marked improvement whereas *vatha* people showed only symptomatic improvement
- Disorientation of *pitha* was found in 38 patients out of which 28 recovered. 2 of the 4 subjects with *kapha* disorientation, and 3 out of 6 subjects with *vatha* disorientation also recovered to normal. This indicates that this plant was more effective in treating *pitha* dominated diabetic complications as compared to *vatha* and *kapha* dominated complications.
- Significant changes in fasting blood sugar were observed only in 28 patients
- 30 subjects showed significant improvement in post prandial glycemic level after treatment.
- Glycated hemoglobin was markedly reduced in 20 patients
- Most of the subjects reported reduction in excessive appetite, which is the most common symptom of Type II Diabetes.

The data of the results obtained clearly depict the anti-diabetic efficacy of ***Nilavembu Kudineer*** in human subjects.

To conclude the study drug, ***Nilavembu Kudineer*** is proved for its quality, both botanically and chemically and is found to be safe, effective and economical in the management of type 2 Diabetes mellitus and its related complications such as nephropathy.

Recommendations

- It is recommended that ***Nilavembu Kudineer*** can be used for adult patients in the management of Diabetes mellitus and its complications.

- This Siddha formulation is also recommended in the cases of Diabetic nephropathy due to its potential in stabilizing renal parameters.

- If ***Nilavembu Kudineer*** is used as hypoglycemic agents, may also reverse dyslipedemia associated with diabetes and prevent the cardiovascular complications which are very prevalent in diabetic patients.

- Further in depth pharmacodynamic and pharmacokinetics studies are needed to understand its bioavailability and release in the biological system. This can contribute in understanding the mechanism of anti-diabetic action leading to global acceptance and recognition of this unique anti-diabetic Siddha formulation.

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